

PRINCIPLES AND PRACTICE OF
AGRICULTURAL ANALYSIS

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Principles and Practice
OF
AGRICULTURAL ANALYSIS

A Manual for the Study of Soils, Fertilizers, and
Agricultural Products

For the Use of Analysts, Teachers, and Students of Agricultural
Chemistry

SECOND EDITION, REVISED AND ENLARGED

VOLUME III.

AGRICULTURAL PRODUCTS

BY HARVEY W. WILEY, A.M., Ph.D.

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PREFACE TO THE SECOND EDITION OF VOLUME THIRD.

The revised edition of Part III. of Principles and Practice has been delayed for several years, by reason of stress of other duties, which made it impossible for the author to bring the matter up to date. To do this the contents of the volume had to be considerably increased. In order that it might not be too large, some of the less important parts of the first edition were necessarily omitted. Even with these omissions it is not possible to conclude the volume with less than 100 pages of extra matter.

In deleting the original matter, endeavor was made not to detract anything from the historical value of the work, nor from the elucidation of the principles on which the practice of the analysis was predicated. The task of revision has, however, now been brought to a close, bringing the matter well up to the close of the year 1913. The author realizes his inability to bring any volume completely up to date. Even while the proofs are read late methods of value are developed, which necessarily can find no place on the printed pages.

The complete revised work, containing more than 2,000 pages, embodies the labors almost of a lifetime devoted to various branches of agricultural research and improvement. Subsequent editions of this work will probably be undertaken by other hands than mine. I simply would urge upon the revisers of the future to retain, if possible, the cardinal principle on which this work is based, namely, to present in as compact a form as possible a connected, historical story of the development of agricultural analysis, emphasizing the fundamental principles on which it is based. If this hope can be realized, this work will be of continuing value to those engaged in agricultural work and in the development of the art of agriculture.

H. W. WILEY.

JANUARY, 1914.

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Volume Third

AGRICULTURAL PRODUCTS

PART FIRST

SAMPLING, DRYING, INCINERATION AND EXTRACTION

1. **Introduction.**—The analyst may approach the examination of agricultural products from various directions. In the first place he may desire to know their proximate and ultimate constitution irrespective of their relations to the soil or to the food of man and beast. In addition to this his study of these products may have reference solely to the determination of the more valuable plant foods which they have extracted from the soil and air. He may also approach his task from a hygienic or economic standpoint for the purposes of determining the wholesomeness or the nutritive and economic values of the products of the field, orchard, or garden. Finally he may pursue his study from a legal standpoint to determine whether these products conform to the state and national laws controlling them. In each case the object of the investigation will have a considerable influence on the method of the examination.

It will be the purpose of the present volume to discuss fully the principles of all the standard processes of analysis and the best practice thereof, to the end that the investigator or analyst, whatever may be the design of his work, may find satisfactory directions for prosecuting it. As in the previous volumes, it should be understood that these pages are written largely for the teacher and the analyst already skilled in the principles of analytical chemistry. Much of detail is therefore left to the individual judgment and experience of the worker, to whom it is hoped a judicious choice of approved processes may be made possible.

2. **Scope of the Work.**—Under the term agricultural products is included a large number of classes of bodies of most

different constitution. In general they are the products of vegetable and animal growth. First of all come the vegetable products, fruits, nuts, grains and grasses. These may be presented in their natural state, such as cereals, grains, fruits and fodders, or after a certain preparation, as starches, sugars and flours. They may also be met with in even more advanced stages of change, as cooked foods, alcohols and secondary organic acids, such as vinegar. In general, by the term agricultural products is meant not only the direct products of the farm, orchard and forest, but also the modified products thereof and the results of manufacture applied to the raw materials. Thus, not only the grain and straw of wheat are proper materials for agricultural analysis, but also flour and bran, bread and cakes made from the grain and paper made from the straw. In the case of maize and barley, still another class of manufactured products arises, for not only do we find starch and malt, but also alcohol and beer falling within the scope of our work. In respect of animal products, the agricultural analyst may be called on to investigate the subject of leather and tanning; to determine the composition of meat, milk and butter; to pass upon the character of lard, oleomargarine and tallow, and, in general, to determine as fully as possible the course of animal food in all its changes between the field, the packing house and the kitchen. The products of the forest are also to be legitimately included in this classification, both directly as nuts, waxes, sugars and gums, and as manufactured products, such as tannins, turpentine, resins, wood-pulp, paper and the products of destructive distillation.

3. Limitations of Work.—It is evident from the preceding paragraph, that in order to keep the magnitude of this work within reasonable bounds the text must be rigidly confined to the fundamental principles and practice of agricultural analysis. The interesting region of pharmacy and allied branches, in respect of plant analysis, can find no description here, and in those branches of technical chemistry, where the materials of elaboration are the products of the field or forest only a superficial view can be given. The main purpose and motive of this volume must relate closely to the more purely agricultural processes.

4. General Manipulations.—There are certain analytical operations which are more or less of a general nature, that is, they are of general application without reference to the character of the material at hand. Among these may be mentioned the determination of moisture and of ash, and the estimation of matters soluble in ether, alcohol and other solvents. These processes will be first described. Preliminary to these analytical steps it is of the utmost importance that the material be properly prepared for examination. In general this is accomplished by grinding or crushing the samples to a fine powder, the attrition being continued until all the particles are made to pass a sieve of given fineness. The best sieve for this purpose is one having circular apertures half a millimeter in diameter. In the process of grinding many bodies are apt to lose moisture. In the case of the grains of Indian corn for example we have found in some cases that the fine ground meal has contained a lower percentage of moisture than the unbroken kernels. In such cases if the original moisture is to be determined it is better to attempt the drying in the crude sample than to run the risk of losing moisture by grinding. Some products, both vegetable and animal, require to be reduced to as fine a state as possible without drying. In such instances, passing the product through a sieve is obviously impracticable. Special grinding and disintegrating machines are made for these purposes and they will be described further on.

There are some agricultural products which have to be prepared for examination in special ways and these methods will be given in connection with the processes for analyzing the bodies referred to. Nearly all the bodies, however, with which the analyst will be concerned, can be prepared for examination by the general methods about to be described.

5. Preparation of the Sample. (a) *Vegetable Substances.*—For all processes of analysis not executed on the fresh sample, substances of a vegetable nature should, if in a fresh state, be dried as rapidly as possible to prevent fermentative changes. It is often of interest to determine the percentage of moisture in the fresh sample. For this purpose a representative portion of the

sample should be rapidly reduced to as fine a condition as possible. To accomplish this it should be passed through a shredding machine, or cut by scissors or a knife into fine pieces. A few grams of the shredded material are dried in a flat bottomed dish at progressively increasing temperatures, beginning at about 60° and ending at from 100° to 110° . The latter temperature should be continued for only a short time. The principle of this process is based upon the fact that if the temperature be raised too high at first, some of the moisture in the interior cells of the vegetable substance can be occluded by the too rapid desiccation of the exterior layers which would take place at a high temperature. The special processes for determining moisture will be given in another place.

The rest of the sample should be partly dried at a lower temperature or air-dried. In the case of fodders and most cattle foods the samples come to the analyst in a naturally air-dried state. When grasses are harvested at a time near their maturity they are sun-dried in the meadows before placing in the stack or barn. Such sun-dried samples are already in a state fit for grinding. Green grasses and fodders should be dried in the sun, or in a bath at a low temperature from 50° to 60° until all danger of fermentative action is over, and then air- or sun-dried in the usual way.

Seeds and cereals usually reach the analyst in a condition suited to grinding without further preliminary preparation. Fruits and vegetables present greater difficulties. Containing larger quantities of water, and often considerable amounts of sugar, they are dried with greater difficulty. The principles which should guide all processes of drying are those already mentioned, viz., to secure a sufficient degree of desiccation to permit of fine grinding and at a temperature high enough to prevent fermentative action, and yet not sufficiently high to cause any marked changes in the constituents of the vegetable or ganism.

(b) *Animal Substance*.—The difficulties connected with the preliminary treatment of animal substances are far greater than those just mentioned. Such samples are composed of widely dif

fering tissues, blood, bone, tendon, muscle and adipose matters, and all the complex components of the animal organism are to be considered. The whole animal may be presented for analysis, in which case the different parts composing it should be separated and weighed as exactly as possible. Where only definite parts are to be examined it is best to separate the muscle, bone, and fat as well as may be, before attempting to reduce the whole to a fine powder. The soft portions of the sample are to be ground as finely as possible in a meat or sausage cutter. The bones are crushed in some appropriate manner, and thus prepared for further examination. Where the flesh and softer portions are to be dried and finely ground, the presence of fat often renders the process almost impossible. In such cases the fat must be at least partially removed by petroleum or other solvent. In practically fat-free samples the material, after grinding in a meat cutter, can be partially dried at low temperatures from 60° to 75°, and afterwards ground in much the same manner as is practiced with vegetable substances.

As is the case with the preliminary treatment of vegetable matters, it is impossible to give any general directions of universal applicability. The tact and experience of the analyst in all these cases are better than any dicta of the books. In some instances, as will appear further on, definite directions for certain substances can be given, but in all cases the general principles of procedure are on the lines already indicated.

6. Preserving Samples.—In most cases, as is directed in the foregoing paragraphs, the sample may be dried before grinding to such a degree as to prevent danger from fermentation or decay. The fine-ground samples are usually preserved in glass stoppered bottles, carefully marked or numbered. In some cases it is advisable to sterilize the bottles after stoppering, by subjecting them to a temperature of 100° for some time. In the case of cereals assurance should be had that the samples do not contain the eggs of any of the pests that often destroy these products. As a rule, samples should be kept for a time after the completion of the analytical work, and this is especially true in all cases where there is any prospect of dispute or litigation. In general

it may be said, that samples should be destroyed only when they are spoiled, or when storage room is exhausted.

7. Collecting Samples.—When possible, the analyst should be his own collector. There is often as much danger from data obtained on non-representative samples as from imperfect manipulation. When personal supervision is not possible, the sample when received, should be accompanied by an intelligible description of the method of getting it, and of what it represents. In all cases the object of the examination must be kept steadily in view. Where comparisons are to be made the methods of collecting must be rigidly the same.

The processes of analysis, as conducted with agricultural products, are tedious and difficult. The absolutely definite conditions that attend the analysis of mineral substances, are mostly lacking. The determinations of carbon, hydrogen, nitrogen and sulfur, which are required in the usual processes of organic analysis, are simplicity itself when compared with the operations which have to be performed on agricultural products to determine their character and their value as food and raiment. *We have to do here with matters in which the sustenance, health and prosperity of the human race are more intimately concerned than with any other of the sciences.* This fact also emphasizes the necessity for care in collecting the materials on which the work is to be performed.

GENERAL PRINCIPLES OF COMMINATION

8. Grinding Samples.—In order to properly conduct the processes of agricultural analysis it is important to have the sample finely ground. This arises both from the fact that such a sample is apt to contain an unevenly distributed content of the various complex substances of which the material under examination is composed, and because the analytical processes can be conducted with greater success upon the finely divided matter. In mineral analysis it is customary to grind the sample to an impalpable powder in an agate mortar. With agricultural products, however, such a degree of fineness is difficult to attain, and moreover, is not necessary. There is a great difference of opin-

ion among analysts respecting the degree of fineness desirable. In some cases we must be content with a sample which will pass a sieve with a millimeter mesh; in fact it may be found impossible, on account of the stickiness of the material, to sift it at all. In such cases a thorough trituration, so as to form a homogeneous mass will have to be accepted as sufficient. Where bodies can be reduced to a powder, however, it is best to pass them through a sieve with circular perforations half a millimeter in diameter. A finer degree of subdivision than this is rarely necessary.

9. The Grinding Apparatus.—The simplest form of apparatus for reducing samples for analysis to a condition suited to passing a fine sieve is a mortar. Where only a few samples are to be prepared and in small quantities, it will not be necessary to provide anything further. After the sample is well disintegrated it is poured on the sieve and all that can pass is shaken or brushed through. The sieve is provided with a receptacle, into which it fits closely, to avoid loss of any particles which may be reduced to a dust. The top of the sieve, when shaken, may also be covered if there be any tendency to loss from dust. Any residue failing to pass the sieve is returned to the mortar and the process thus repeated until all the material has been secured in the receiver. The particles more difficult of pulverization are often different in structure from the more easily pulverized portions, and the sifted matter must always be carefully mixed before the subsample is taken for examination. Often the materials, or portions thereof, will contain particles tough and resistant to the pestle, but the operator must have patience and persistence, for it is highly necessary to accurate work that the whole sample be reduced to proper size.

Where many samples are to be prepared, or in large quantities, mills should take the place of mortars. For properly air-dried vegetable substances, some form of mill used in grinding drugs may be employed. Grinding surfaces of chilled corrugated steel are to be preferred. The essential features of such a mill are that it be made of the best material, properly tempered, and that the parts be easily separated for convenience in clean-

ing. The grinding surfaces must also be so constructed and adjusted as to secure the proper degree of fineness. In Fig. 1 is shown a mill of rather simple construction, which has long been in satisfactory use. Small mills may be operated by hand power, but when they are to be used constantly other power should be provided. In addition to the removal of nearly all the moisture by air-drying there are many oleaginous seeds which cannot be finely ground until their oil has been removed. For this purpose the grinding surfaces of the mill are opened

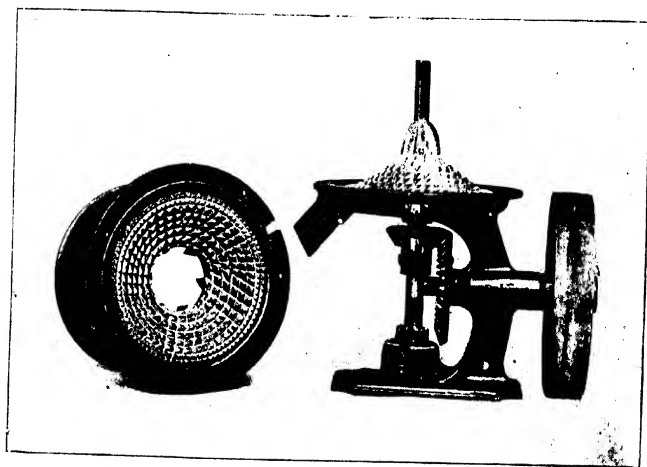


Fig. 1.—Mill for Grinding Dry Samples.

so that the seeds are only coarsely broken in passing through. The fragments are then digested with light petroleum in a large flask, furnished with a reflux condenser. After digestion and separation of the extracted oil the fragments are again passed through the mill adjusted to break them into finer particles.

The alternate grinding and digestion are thus continued until the pulverization is complete. On a small specially prepared sample the total content of oil is separately determined.

Fresh animal tissues are best prepared for preliminary treatment by passing through a sausage mill. The partially homo-

genous mass thus secured should be dried at a low temperature and reground as finely as possible. Where much fat is present it may be necessary to extract it as just mentioned, in the case of oleaginous seeds. In such cases both the moisture and fat in the original material should be determined on small specially prepared samples with as great accuracy as possible. Bones, hoofs, horns, hair and hides present special difficulties in preparation, which the analyst will have to overcome with such skill and ingenuity as he may possess.

The analyst will find many specially prepared animal foods already in a fairly homogenous form, such as potted and canned meats, infants' and invalids' foods, and the like. Even with these substances, however, a preliminary grinding and mixing will be found of advantage before undertaking the analytical work. Many cases will arise which apparently do not fall within the classification given above. But even in such instances the analyst should not be without resources. Frequently some dry inert substance may be mixed with the material in definite quantities, whereby it is rendered more easily prepared. Perhaps no case will be presented where persistent and judicious efforts to secure a fairly homogeneous sample for analysis will be wholly unavailing.

In the case of green vegetable matters which require to be reduced rapidly to a fine state of subdivision in order to secure even a fairly good sample some special provision must be made. This is the case with stalks of maize and sugar-cane, root crops, such as potatoes and beets, and green fodders, such as clover and grasses. The chopping of these bodies into fine pieces by hand is slow and often impracticable. The particles rapidly lose moisture and it is important to secure them promptly as in the preparation of beet pulp for polarization. For general use we have found the apparatus shown in Fig. 2 quite satisfactory. It consists of a series of staggered circular saws carried on an axis and geared to be driven at a high velocity, in the apparatus mentioned at 1,400 revolutions per minute. The green material is fed against the revolving saws by the toothed gear-work shown, and is thus reduced to a very fine pulp, which is re-

ceived in the box below. Stalks of maize, green fodders, sugar-canes, beets and other fresh vegetable matters are by this process reduced to a fine homogeneous pulp, well suited for sampling and for analytical operations. Such pulped material can also be spread in a fine layer and dried rapidly at a low temperature, thus avoiding danger of fermentative changes when it is desired to secure the materials in a dry condition or to preserve them for future examination. Samples of sorghum cane, thus

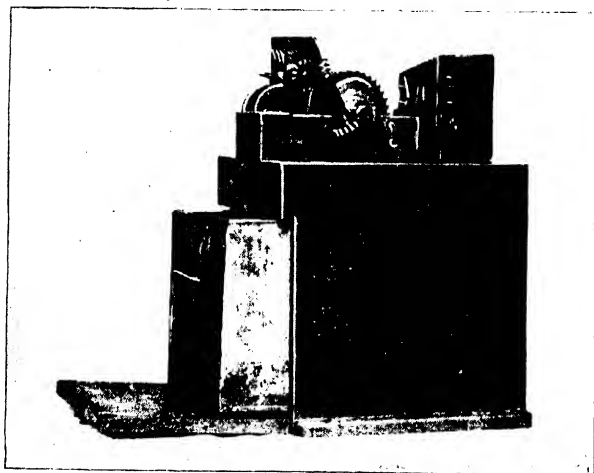


Fig. 2. Comminutor for Green Samples. (Top removed).

pulped and dried, have been preserved for many years with their sugar content unchanged.

Such a machine is also useful in preparing vegetable matter for the separation of its juices in presses. Samples of sugar cane, sugar-beets, apples and other bodies of like nature can thus be prepared to secure their juices for chemical examination. Such an apparatus we have found is fully as useful and indispensable in an agricultural laboratory as a drug mill for air-dried materials. It is known as the Hyatt shredder.

It is often desirable in the preparation of roots for sugar

analysis to secure them in a completely disintegrated state, that is with the cellular tissues practically all broken. Such a pulped material can be treated with water and the sugar juices it contains thus at once distributed to all parts of the liquid mass. The operation is known as instantaneous diffusion. The pulp of the vegetable matter is thus introduced into the measuring flask along with the juices and the content of sugar can be easily determined. Several forms of apparatus have been devised for this purpose, one of which is shown in Fig. 3. This

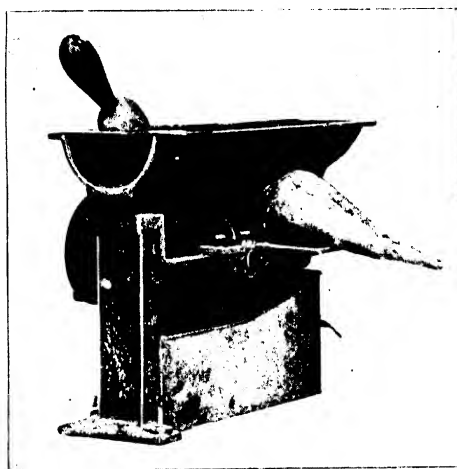


Fig. 3.- Rasp for Sugar Beets.

process, originally devised by Pellet, has come into quite general use in the determination of the sugar content of beets.¹ It is observed that it can be applied to other tubers, such as the turnip, potato, artichoke, etc. It is desirable, therefore, that an agricultural laboratory be equipped with at least three kinds of grinding machines: viz., first, the common drug mill used for grinding seeds, air-dried fodders, and the like; second, a pulping machine like the system of staggered saws above described

¹ Sidersky, *Traité d'Analyse des Matières Sucrées*: 311.

for the purpose of reducing green vegetable matter to a fine state of subdivision, or one like the pellet rasp for tubers; third, a mill for general use such as is employed for making sausages from soft animal tissues.

10. Grinding Apparatus at Halle Station.—The machine used at the Halle station for grinding samples for analysis is shown in Fig. 4.² It is so adjusted as to have both the upper and lower

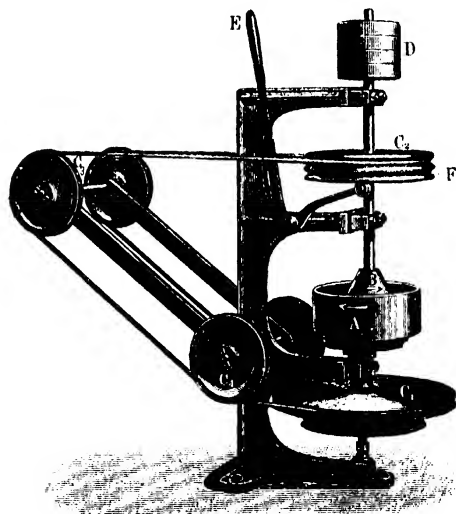


Fig. 4.—Dreef Grinding Apparatus.

grinding surfaces in motion. The power is transmitted through the pulley D, which is fixed to an axis carrying also the inner grinding attachment B. Through C₂, C₃, C₄, and C₁, the reverse motion is transmitted to the outer grinder A. By means of the lever E the two grinding surfaces can be separated when the mill is to be cleaned. The Dreef mill above described is especially useful for grinding malt, dry brewers' grains, cereals for starch determinations and similar dry bodies. It is not suited to grind-

² Bieler and Schneidewind, Die agricultur-chemische Versuchs-Station, Halle a/S: 11.

ing oily seeds and moist samples. These, according to the Halle methods, are rubbed up in a mortar until of a size suited to analysis, and samples such as moist residues, wet cereals, mashes, beet cuttings, silage, etc., are dried before grinding. If it be desired to avoid the loss of acids which may have been formed during fermentation, about ten grams of magnesia should be thoroughly incorporated with each kilogram of the material before drying.

11. Preliminary Treatment of Fish.—The method used by Atwater in preparing fish for analysis is given below.³ The same process may also be found applicable in the preparation of other animal tissues. The specimens, when received at the laboratory, are at once weighed. The flesh is then separated from the refuse and both are weighed. There is always a slight loss in the separation, due to evaporation and to slimy and fatty matters and small fragments of the tissues which adhere to the hands and the utensils employed in preparing the sample. Perfect separation of the flesh from the other parts of the fish is difficult, but the loss resulting from imperfect separation is small. The skin of the fish, although it has considerable nutritive value, should be separated with the other refuse.

The partial drying of the flesh for securing samples for analytical work is accomplished by chopping it as finely as possible and subjecting from fifty to one hundred grams of it for a day to a temperature of 96° C. in an atmosphere of hydrogen. After cooling and allowing to stand in the open air for 12 hours, the sample is again weighed, and then ground to a fine powder and made to pass a sieve with a half millimeter mesh. If the samples are very fat they cannot be ground to pass so fine a sieve. In such a case a coarser sieve may be used or the sample reduced to as fine and homogeneous a state as possible, and bottled without sifting.

The reason for drying in hydrogen is to prevent oxidation of the fats. As will be seen further on, however, such bodies can be quickly and accurately dried at low temperatures in a vacuum, and thus all danger of oxidation be avoided. In fact, the pre-

³ Report of Commissioner of Fish and Fisheries, 1888:687.

liminary drying of all animal and vegetable tissues, where oxidation is to be feared, can be safely accomplished in a partial vacuum by methods to be described in another place. In order to be able to calculate the data of the analysis to the original fresh state of the substance, a portion of the fresh material should have its water quantitatively determined as accurately as possible.

DRYING ORGANIC BODIES

12. Volatile Bodies.—In agricultural analysis it becomes necessary to determine the percentage of bodies present in any given sample which is volatile at any fixed temperature. The temperature reached by boiling water is the one which is usually selected. It is true that this temperature varies with the altitude, and within somewhat narrow limits at the same altitude due to variations in barometric pressure. As the air pressure to which any given body is subjected, however, is a factor in the determination of its volatile contents, it will be seen that within the altitudes at which chemical laboratories are generally found, the variations in the boiling-point of water will not be important. This arises from the fact that while water boils at a lower temperature, as the height above the sea level increases, the corresponding diminished air pressure permits a more ready escape of volatile matter. As a consequence, a body dried to constant weight at sea level, where the temperature of boiling water is 100° , will show practically the same percentage of volatile matter as if dried at an altitude where water boils at 99° . When, therefore, it is desirable to determine the volatile matter approximately at 100° in a sample, it is better to direct that it be done in a space surrounded by steam at the natural pressure rather than at exactly 100° , a temperature somewhat difficult to constantly maintain. However, where it is directed or desired to dry to constant weight exactly at 100° , it can be accomplished by means of an air-bath or by a water-jacketed-bath under pressure, or to which enough solid matter is added to raise the boiling-point to 100° . It is not often, however, that it is worth while to make any special efforts to secure a temperature of exactly 100° . When bodies are to be dried at temperatures above

100°, such as 105°, 110°, and so on, an air-bath is the most convenient means of securing the desired end. The different kinds of apparatus to be employed will be described in succeeding paragraphs.

13. Drying at the Temperature of Boiling Water.—The best apparatus for this process is so constructed as to have an interior space entirely surrounded with boiling water or steam, with the

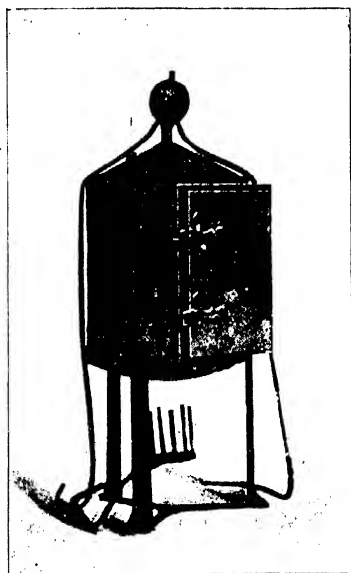


Fig. 5.—Water-Jacketed Drying Oven.

exception of the door by which entrance is gained thereto. The metal parts of the apparatus are constructed of copper, and to keep a constant level of water and avoid the danger of evaporating all the liquid, it is advisable to have a reflux condenser attached to the apparatus. It is also well to secure entrance to the interior drying oven, not only by the door, but also by small circular openings, which serve both to hold a thermometer and

to permit of the aspiration of a slow stream of dry air through the apparatus during the progress of desiccation. The gaseous bodies formed by the volatilization of the water and other matters are thus carried out of the drying box and the process thereby accelerated. The bath should be heated by a burner so arranged as to distribute the flame as evenly as possible over the base. A single lamp, while it will boil the water in the center, will not keep it at the boiling-point on the sides. The temperature of the interior of the bath will not therefore reach 100° . The interior of the oven should be coated with a non-detachable carbon paint to promote the radiation of the heat from its walls as well as to protect the parts from oxidation when acid fumes are produced during desiccation. Instead of using a reflux condenser a constant water level may be maintained in the bath by means of a mariotte bottle or other similar device.

When a bath of this kind is arranged for use with a partial vacuum, it should be made cylindrical in shape, with conical ends, as shown in Fig. 5, in order to bear well the pressure to which it is subjected. Among the many forms of steam-baths offered, the analyst will have but little difficulty in selecting one suited to his work. To avoid radiation the exterior of the apparatus should be covered with a non-conducting material or protected by an air space.

14. Drying in a Closed Water Oven.—When it is desired to keep the temperature of a drying oven exactly at 100° instead of at the heat of boiling water, a closed water oven with a thermostat is to be employed. The oven should be so constructed as to secure a free circulation of the water about the inner space. Since as a rule the water between the walls of the apparatus will be subjected to a slight pressure, these walls should be made strong, or the cylindrical form of apparatus should be used. The thermostat used by the Halle Station is shown in Fig. 6.⁴ A U shaped tube, with a bulb on one arm and a lateral smaller tube sealed on the other, is partly filled with mercury and connected by rubber tubes on the right with the gas supply, and on the left

⁴ Bieler and Schneidewind, Die agricultur-chemische Versuchs-Station, Halle a.S. : 14.

with the burner. The end carrying the bulb is connected directly by a rubber and metal tube with the water space of the oven. This device is provided with a valve which is left open until the temperature of the drying space reaches about 95° . The tube conducting the gas is held in the long arm of the U by means of a cork through which it passes air-tight and yet is loose enough to permit of its being moved. Its lower end is provided with a long Λ shaped slit. When the valve leading to the water space is closed and the water reaches the boiling-point, the pressure of

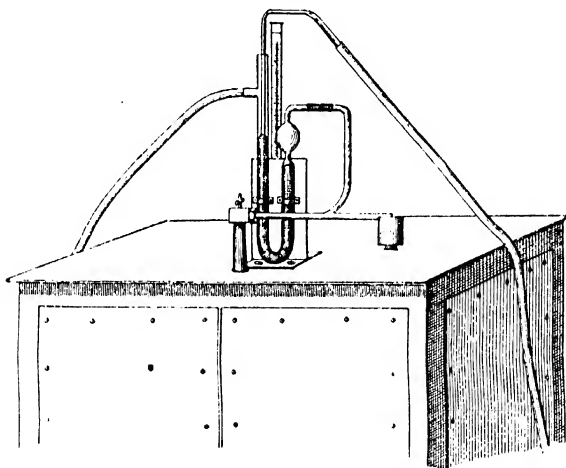


Fig. 6.—Thermostat for Steam-Bath.

the vapor depresses the mercury in the bulb arm of the U and raises it in the other. As the mercury rises it closes the wider opening of the Λ shaped slit; thus diminishing the flow of gas to the burner. By moving the gas entry tube up or down a position is easily found in which the temperature of the drying space, as shown by the thermometer, is kept accurately and constantly at 100° .

In a bath arranged in this way a steam condenser is not necessary. Since, however, in laboratories which are not at a higher altitude than 1,000 feet the boiling-point of water is nearly

100°, it does not seem necessary to go to so much trouble to secure the exact temperature named. There could be no practical difference in the percentage of moisture determined at 100°, and at the boiling-point of water at a temperature not more than 1° lower.

15. Drying in an Air-Bath.—In drying a substance in a medium of hot air surrounded by steam, as has been described, the process is, in reality, one of drying in air. The apparatus usually meant by the term air-bath, however, has its drying space heated directly by a lamp, or indirectly by a stratum of hot air occupying the place of steam in the oven already described. The simplest form of the apparatus is a metal box, usually copper, heated from below by a lamp. In the jacketed forms the currents of hot air produced directly or indirectly by the lamp are conducted around the inner drying oven, thus securing a more even temperature. The bodies to be dried are held on perforated metal or asbestos shelves in appropriate dishes, and the temperature to which they are subjected is determined by a thermometer, the bulb of which is brought as near as possible to the contents of the dish. One advantage of the air-bath is in being able to secure almost any desired temperature from that of the room to one of 150° or even higher. Its chief disadvantage lies in the difficulty of securing and maintaining an even temperature throughout all parts of the apparatus. Radiation from the sides of the drying oven should be prevented by a covering of asbestos or other non-combustible and non-conducting substance. The burner employed should be a broad one and give as even a distribution of the heat as possible over the bottom of the apparatus.

16. Spencer's Air-Drying Oven.—In order to secure an even distribution of the heat in the desiccating space of the oven. Spencer has devised an apparatus, shown in the figure, in which the temperature is maintained evenly throughout the apparatus by means of a fan.⁵ The oven has a double bottom, the space between the two bottoms being filled with air. The sides are also double, the space between being filled with plaster. The

⁵ Journal of the American Chemical Society, 1898, 15:82.

fan is driven by a toy engine connected with the compressed air service or other convenient method. Thermometers placed in different parts of the apparatus, while in use, show rigidly even heat at all points so long as the fan is kept in motion.

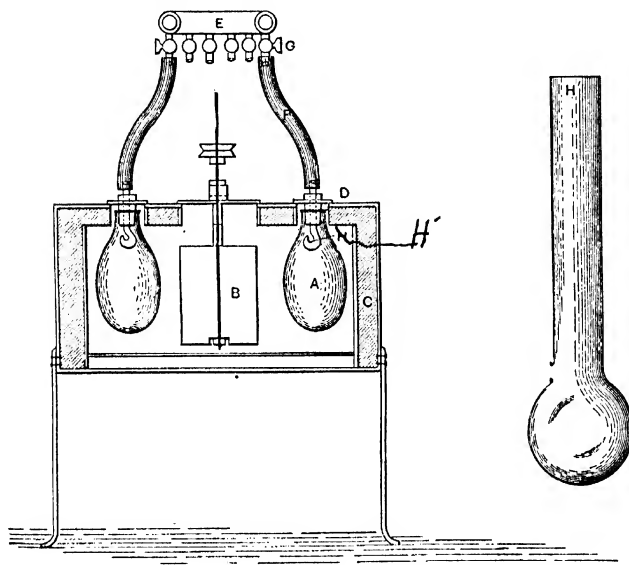


Fig. 7.—Spencer's Drying Oven.

The actual temperature desired can be controlled by a gas regulator. This form of apparatus is well suited to drying a large number of samples at once.

17. Drying in Bulbs.—Spencer's oven can also be used to advantage in drying viscous liquids in a partial vacuum. For this purpose the flask A, Fig. 7, containing the substance is made with a round bottom to resist the atmospheric pressure. Its capacity is conveniently from 150 to 200 cubic centimeters. It is closed with a rubber stopper carrying a trap, H, to keep the evaporated water from falling back. The details of the construction of the trap H are shown at the right of the figure. The vapors enter at the lateral orifice, just above the bulb, while the condensed

water falls back into the bulb instead of into the flask A. Where but little water is condensed the trap may be simply a curved tube with a large belly as shown in H'. A series of flasks can be used at once connected through the stop-cocks G with the circular tube E leading to the vacuum. A water-pump easily exhausts the apparatus, maintaining a vacuum of about 27 inches. The hot air in the oven is kept in motion by the fan B, thus ensuring an even temperature in every part. The flask A may be partly filled with sand or pumice stone before the addition of the samples to be dried, and the weight of water lost is determined by weighing A before and after desiccation. If it be desired to introduce a slow current of dry air or some inert gas into A, it is easily accomplished by passing a small tube, not shown in the figure, connected with the dry air or gas supply, through the rubber stopper and extending it into the flask as far as possible without coming into contact with the contents.

18. Drying Under Diminished Air Pressure—The temperature at which any given body loses its volatile products is conditioned largely by the pressure to which it is subjected. At an air pressure of 760 millimeters of mercury, water boils at 100° but it is volatilized at all temperatures. As the pressure diminishes the temperature at which a body loses water at a given rate falls. This is a fact of importance to be considered in drying many agricultural products, and particularly true of those containing large quantities of oils and sugars. Invert sugar especially is apt to suffer profound changes at a temperature of 100°, the levulose it contains undergoing partial decomposition at high temperatures in the presence of oxygen.

In drying in a partial vacuum therefore a double advantage is secured, that of a lower temperature of desiccation and in the presence of less oxygen. It is not necessary to have a complete vacuum. There are few organic products which can not be completely deprived of their volatile matters at a temperature of from 70° to 80° in a partial vacuum in which the air pressure has been diminished to about one-quarter or less of its normal force.

19. Electric Drying Bath.—The heat of an electric current can be conveniently used for drying in a partial vacuum by means of

the simple device illustrated in Fig. 8. In ordering a heater of this kind the voltage of the current should be stated. The current generally in use has a voltage of about 110, and is installed on the three wire principle. It is well to use a rheostat with the heater in order to control the temperature within the bell

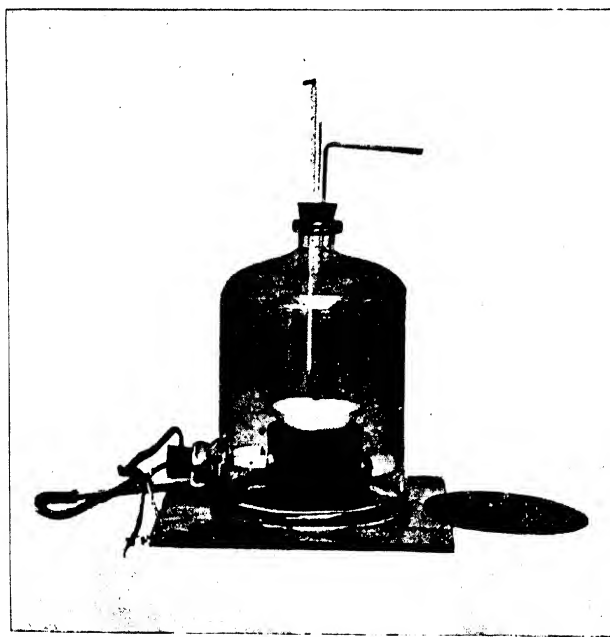


Fig. 8.—Electric Vacuum Drying Oven.

jar. The ground rim of the bell jar rests on a rubber disk placed on a thick ground glass or a metal plate, making an airtight connection. A disk of asbestos may serve to separate the heater from the dish containing the sample, in cases where a high temperature is to be avoided.

20. Steam Coil Apparatus.—For drying at the temperature of superheated steam, it is convenient to use an apparatus furnished with layers or coils of steam-pipes. The drying may be

accomplished either in the air or in a vacuum. A large drying oven, having three sets of brass steam-tubes and sides of non-conducting material, is employed with great advantage. The series of heating pipes is so arranged as to be used one at a time or collectively. Each series is furnished with a separate steam valve, and is provided with a trap to control the escape of the condensed vapors. In the bottom of the apparatus are apertures through which air can enter, which after passing through the interior of the oven escapes through a ventilator at the top. With a pressure of forty pounds of steam to the square inch and a free circulation of air, the temperature on the first shelf of the apparatus is about 98° ; on the second from 103° to 104° , and on the third about 100° . The vessels containing the bodies to be dried are not placed directly on the brass steam-pipes, but the latter are first covered with thick perforated paper or asbestos. For drying large numbers of samples, or large quantities of one sample, such an apparatus is almost indispensable to an agricultural laboratory.

A smaller apparatus is shown in Fig. 9. The heating part G is made of a small brass tube arranged near the bottom in a horizontal coil and continued about the sides in a perpendicular coil. Bodies placed on the horizontal shelf are thus entirely surrounded by the heating surfaces except at the top.⁶ The steam-pipe S is connected with the supply by the usual method, and the escape of the condensation is controlled either by a valve or trap in the usual way. The whole apparatus is covered by a bell jar B, resting on a heavy cast-iron plate P, through which also the ends of the brass coil pass. The upper surface of the iron plate may be planed, or a planed groove may be cut into it, to secure the edge of the bell jar. When the air is to be exhausted from the apparatus, a rubber washer should be placed under the rim of the bell jar. The latter piece of apparatus may either be closed, as shown in the figure, by a rubber stopper, or it is better, though not shown, to have a stopper with three holes. One tube passes just through the stopper and is connected with the vacuum: the second passes to the bottom of the apparatus and serves to

⁶ Division of Chemistry, Bulletin 28 : 101.

introduce a slow stream of dry air or of an inert gas during the desiccation. The third hole is for a thermometer. When no

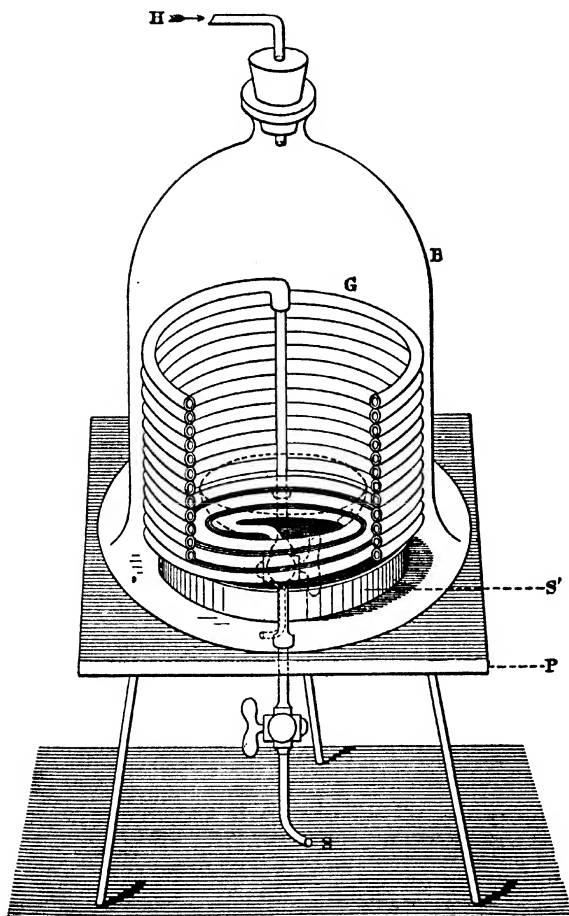


Fig. 9.—Steam Coil Drying Oven.

movement of the residual gas in the apparatus is secured, a dish containing strong sulfuric acid S' is placed on the iron plate and

under the horizontal coil, as is shown in the figure. The sulfuric acid so placed does not reach the boiling-point of water, and serves to absorb the aqueous vapors from the residual air in the bell jar. By controlling the steam supply the desiccation of a sample can be secured in the apparatus at any desired temperature within the limit of the temperature of steam at the pressure used. Where no steam service is at hand a strong glass flask may be used as a boiler, in which case the trap end of the coil must be left open. The vacuum may be supplied by an air or bunsen pump. When a vacuum is not used an atmosphere of dry hydrogen may be supplied through II.

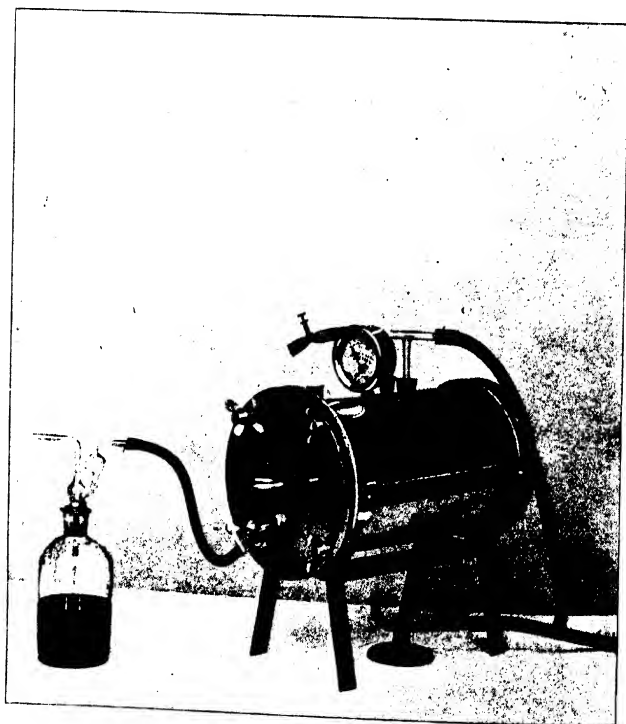


Fig. 10. Carr's Vacuum Drying Oven. (Closed).

21. Carr's Vacuum Oven.—A convenient and very useful drying oven has been devised by Carr and Sanborn.⁷ It is made of a large tube, preferably of brass. The tube may be from six to nine inches in diameter and from twelve to fifteen inches long.



Fig. 10 (Bis).— Vacuum Oven Open.

One end is closed air-tight by a brass end-piece attached by a screw, or brazed. The other end is detachable and is made air-tight by ground surfaces and a heat resisting washer. In the

⁷ Division of Chemistry, Bulletin 47: 134.

figure this movable end-piece is shown attached by screw-nuts, but experience has shown that these are not necessary. On the upper longitudinal surfaces are apertures for the insertion of a vacuum gauge and for attachment to a vacuum apparatus.

In the figure the thermometer and aperture for introducing dry air or an inert gas are shown in the movable end disk, but they would be more conveniently placed in the fixed end. The oven is heated below by a gas burner, which preferably should be as long as the oven. The heat is not allowed to strike the brass cylinder directly, but the latter is protected by a piece of asbestos paper.

The temperature inside of the oven can be easily kept practically constant by means of a gas regulator, not shown in the figure, or by a little attention to the lamp. For a vacuum of twenty inches a temperature of about 80° should be maintained. When the vacuum is more complete a lower temperature can be employed. This apparatus is simple in construction, strong, cheap, and highly satisfactory in use.

22. Drying in Hydrogen.—In some of the processes of agricultural analysis it becomes important to dry the sample in hydrogen or other inert gas. This may be accomplished by introducing the dry gas desired into some form of the apparatus already described. The drying may either be accomplished in an atmosphere of hydrogen practically at rest or in a more limited quantity of the gas in motion. The latter method is to be preferred by reason of its greater rapidity. The analyst has at his command many forms of apparatus designed for the purpose mentioned above. It will be sufficient here to describe only two, devised particularly for agricultural purposes.

The first one of these, designed by the author, was intended especially for drying samples of fodders for analysis.⁸

For the purpose of drying materials contained in flasks and tubes in a current of hydrogen the apparatus shown in Fig. 11 is used. This apparatus consists of a circular box, B, conveniently made of galvanized iron, having a movable cover, fitted for the introduction of steam S, into the interior of the apparatus.

⁸ Division of Chemistry, Bulletin 28 : 100.

Condensed steam escapes at W. A stream of perfectly pure and dry hydrogen enters at H, passes up through the material to be dried, down through the bulb V, containing sulfuric acid, and follows the direction of the arrows through the rest of the

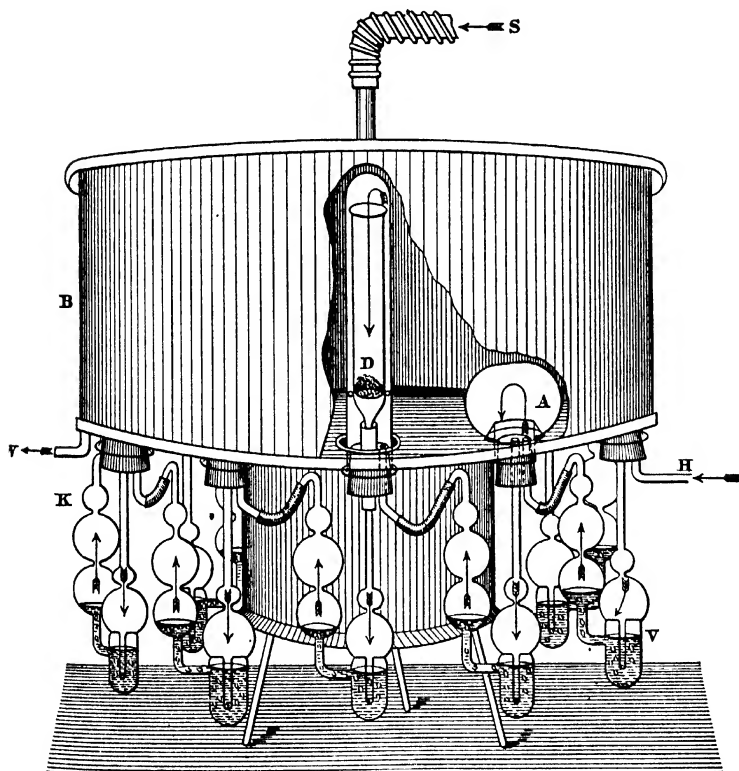


Fig. 11.—Apparatus for Drying in a Current of Hydrogen.

apparatus. The stream of hydrogen is thus completely dried by passing through bulbs containing sulfuric acid, on the way from one piece of the apparatus to the other. A, represents a flask such as is used, with the extraction apparatus described. The apparatus which we have used will hold eight tubes or flasks

at a time, and thus a single stream of hydrogen is made to do duty eight times in drying eight separate samples. The great advantage of the apparatus is in the fact that the stream of hydrogen must pass over and through the substance to be dried. In order to prevent any sulfuric acid from being carried forward into the next tube the bulb K, above the sulfuric acid, may be filled with solid pieces of soda or potash.

This apparatus has been in use for a long time and no accidents from sulfuric acid being carried forward have occurred, and there is no danger, provided the stream of hydrogen is kept running at a slow rate. If, however, by any accident the stream of hydrogen should be admitted with great rapidity, particles of the sulfuric acid might be carried forward and spoil the next sample. To avoid any such accident as this the proposal to introduce the potash bulb has been made. The apparatus works with perfect satisfaction, and it is believed that when properly adjusted check weighings can be made by weighing the bulbs, showing their increase in weight, which will give the volatile matter, and weighing the flasks or tubes, which will show the loss of weight. The only chance for error in weighing the bulbs is that some of the volatile matter may be material which is not dissolved in sulfuric acid, and is thus carried on and out of the apparatus. The blackening of the sulfuric acid in the bulbs, in the drying of all forms of organic matter, shows that the loss in weight of such bodies is not due to water alone, but also to organic volatile substances, which are capable of being decomposed by the sulfuric acid, thus blackening it.

23. Caldwell's Hydrogen Drying-Bath.—An excellent device for drying in hydrogen has been described by Caldwell.⁹ A vessel of copper or other suitable material serves to hold the tubes containing the samples to be dried. It should be about twenty-four centimeters long, fifteen high, and eight wide. This vessel is contained in another made of the same material and of the dimensions shown in the figure. On one side the edge of this containing vessel may not be more than one centimeter high and

⁹ Cornell University, Agricultural Experiment Station, Chemical Department, Bulletin 12:147.

the bath should rest against it. The other side is made higher to form a support for the drying tubes as indicated.

The tube containing the substance *a d* is made of glass and may be closed by the ground stoppers *c b* or the tube stoppers *e f*. At *a* it carries a perforated platinum disk for holding the filtering felt. The tube should be about 13 centimeters long and have an internal diameter of about 20 millimeters. With its

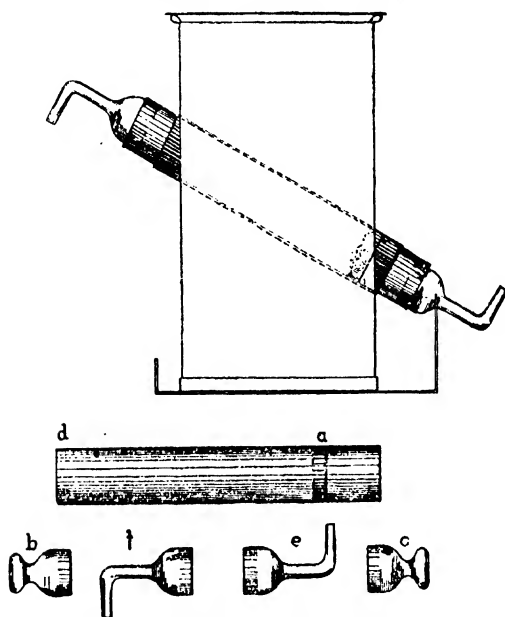


Fig. 12.—Caldwell's Hydrogen Drying Apparatus.

stoppers it should weigh only a little over 30 grams. The asbestos felt should not be thick enough to prevent the free passage of gas. Passing diagonally through the bath are metal tubes, preferably made of copper, and of such a size as just to receive the glass drying tubes. If these be a little loose they should be made tight by wrapping them with a narrow coil of paper at either end of the tubular receptacle. The entrance of cold air between the glass tube and its metal holder is thus pre-

vented, and the glass tube is held firmly in position. The glass tube should be weighed with its two solid stoppers. Afterwards the sample, about two grams, is placed on the asbestos felt and the stoppers replaced and the whole reweighed. The exact weight of the sample is thus obtained. The solid stoppers are then removed and the tube stoppers inserted. The lower end of the tube is then connected with the supply of dry hydrogen. The upper tube stopper is connected by a rubber tube with a small bottle containing sulfuric acid through which the escaping hydrogen is made to bubble. A double purpose is thus secured; moisture is kept from entering the drying tube and the rate at which the hydrogen is passing is easily noted. After the drying is completed the solid stoppers are again inserted, the tube cooled in a desiccator and weighed. Precautions should be observed to see that the tubes are filled with dry air before weighing. The loss of weight is entered as water. The tube containing the sample can afterwards be put into an extractor and treated with ether or petroleum in the manner hereafter described. This apparatus requires more hydrogen than the one previously described, but it is rather simple in construction, is easily controlled, and has given satisfactory results.

24. Drying in Liebig's Tubes.—In drying samples, especially of fodders, the method practiced at the Halle Station is to place them in drying tubes, the form of which is shown in Fig. 13.

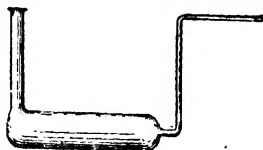


Fig. 13. Liebig's Tube.

A stream of illuminating gas, previously dried by passing over sulfuric acid and calcium chlorid, is directed through the tubes.¹⁰ Many of these tubes can be used at once, arranged as shown in Fig. 14. When the air is all driven out the stream of gas can be ignited so as to regulate the flow properly by the size of the

¹⁰ Die agricultur-chemische Versuchs-Station, Halle a/S: 15.

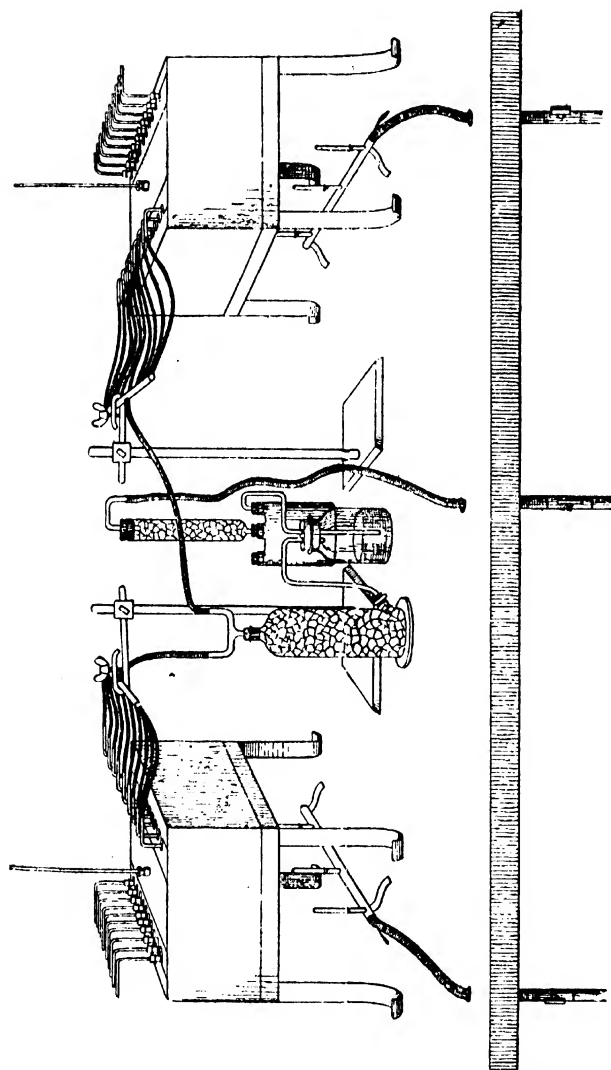


Fig. 14. — Drying Apparatus used at the Halle Station.

flame. The tubes are held in drying ovens, as shown in the figure, the temperature of which should be kept at 105° – 107° . The drying should be continued for eight or ten hours. At the end of this time the gas in the tube is to be expelled by a stream of dry air and the tubes cooled in a desiccator and weighed. There are few advantages in this method not possessed by the processes already described. The samples, moreover, are not left in a condition for further examination, either by incineration or extraction.

25. Wrampelmeyer's Drying Oven.—The apparatus used at the Wageningen Station, in Holland, for drying agricultural

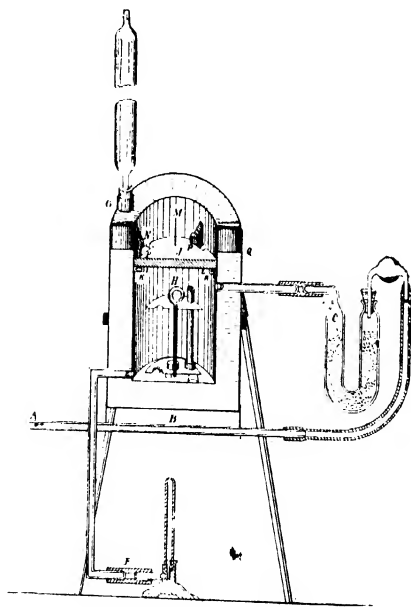


Fig. 15.—Wrampelmeyer's Oven.

samples, was devised by Wrampelmeyer and is shown in Fig. 15.¹¹ The oven is so constructed as to permit of driving a stream of inert gas. Illuminating gas is let into the space of the oven through the tube *a b*. At *b* the gas

¹¹ Die landwirtschaftlichen Versuchs-Stationen, 1891, 38: 1.

is heated by the same lamp which boils the liquid in the water space of the apparatus. The hot gas is dried in the calcium chlorid tube C and then passes into the oven at D. At E it leaves the apparatus and is thence conducted to the lamp F, used for heating the bath. The lamp should be closed by a wire gauze diaphragm to prevent any possible explosion by reason of any admixture with the air in the oven. The condensation of the aqueous vapors is affected by means of the condenser G. In the drying space is a small shelf holder, which, by means of the hook, H, can be removed from the apparatus. The drying space is closed from the upper part of the apparatus, which contains no water, by the cover J, resting on a support K. This rim is covered with a rubber gasket L, by means of which the cover J can be fastened with a bayonet latch air-tight. This fastening is shown at N. Being closed in this way the part of the cylindrical oven above the cover may be left entirely open. Instead of the rather elaborate method of closing the bath, some simple and equally effective device might be used. The cover J is best made with double metallic walls enclosing an asbestos packing.

It is evident that this oven could be used with an atmosphere of carbon dioxid or of air, provided the gas for heating were derived from a separate source and the tube between E and F broken. In a drying oven designed by the author, the movable top is made with double walls and the space between is joined to the steam chamber by means of a flexible metallic tube, thus entirely surrounding the drying space with steam.

26. The Ulsch Drying Oven.—A convenient drying oven is described by Ulsch which varies from the ordinary form of a water-jacketed drying apparatus in having a series of drying tubes inserted in the water-steam space.¹²

The arrangement of the oven is shown in the accompanying figure. The water space is filled only to about one-third of its height. When the heat is applied the cock *c* is left open until the steam has driven out all the air. It is then closed and the temperature of the bath is well regulated by the manometer *e*, connected with the bath by *d*. The bottom of the manometer

¹² *Chem. Zentr.-Zeitung*, 1895, 19: 1183.

cylinder contains enough mercury to keep the end of the manometer tube always sealed. The rest of the space is filled with water. At the top the manometer tube is expanded into a small bulb which serves as a gas regulator, as shown in the figure. The gas is admitted also by a small hole above the mercury in the bulb, so that when the end of the gas inlet tube is sealed

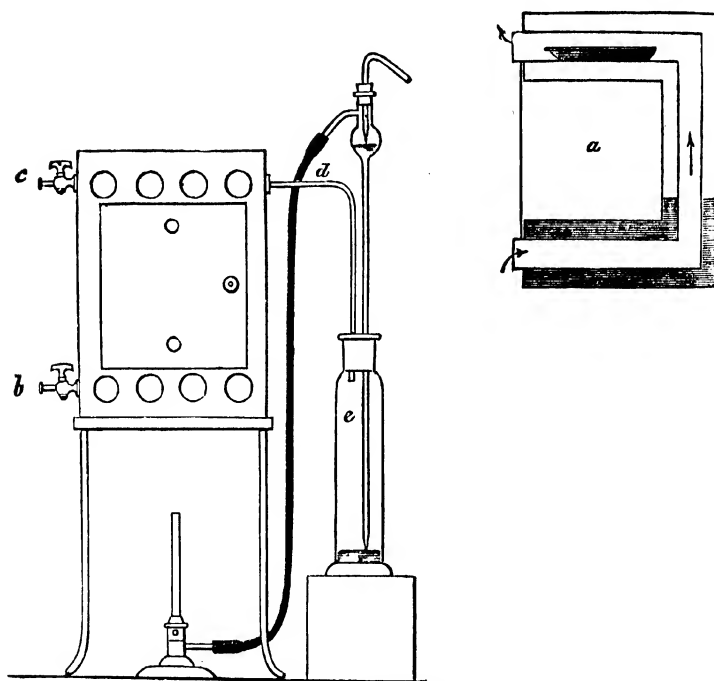


Fig. 16. Ulsch Drying Oven.

enough gas still passes through to keep the lamp burning. With a mercury pressure of 30 centimeters the temperature of the bath will be about 105° . The walls of the bath should be made strong enough to bear the pressure corresponding to this degree. The drying can be accomplished either in the cubical drying box *a* or in the drying tubes made of thin copper and disposed as shown in the figure. The natural draft is shown by

the arrows. The substance is held in boats placed in the tube as indicated. The air in traversing the tube is brought almost to the temperature of the water-steam space in which the tube lies. The natural current of hot air can easily be replaced by a stream of dry illuminating or other inert gas.

27. Drying Viscous Liquids.—In the case of cane juices, milk, and similar substances, the paper coil method may be used.¹³

The manipulation is conducted as follows: A strip of filtering paper from five to eight centimeters wide and 40. centimeters in length, is rolled into a loose coil and dried at the temperature of boiling water for two hours, placed in a dry glass stoppered weighing tube, cooled in a desiccator and weighed. The stoppered weighing tube prevents the absorption of hygroscopic moisture. About three cubic centimeters of the viscous or semi-viscous liquid are placed in a flat dish covered by a plate of thin glass and weighed. The coil is then placed on end in the dish, and the greater part of the liquid is at once absorbed. The proportions between the coil and the amount of liquid should be such that the coil will not be saturated more than two-thirds of its length. It is then removed and placed dry end down in a steam-bath and dried two hours. The dish, covered by the same plate of glass, is again weighed, the loss in weight representing the quantity of liquid absorbed by the coil. After drying for the time specified the coil is again placed in the hot weighing tube, cooled and its weight ascertained. The increase represents the solid matter in the sample taken. This method has been somewhat modified by Josse, who directs that it be conducted as follows:¹⁴ Filter-paper is cut into strips from one to two centimeters wide and three meters long. The strips are crimped so they will not lie too closely together and then wrapped into coils. These coils can absorb about 10 cubic centimeters of liquid. One of them is placed in a flat dish about two centimeters high and seven in diameter, and dried as described, covered, cooled and weighed. There are next placed in the dish and

¹³ Division of Chemistry, Bulletin 13, part one: 85.

¹⁴ Bulletin de l'Association des Chimistes de Sucrerie et de Distillerie, 1893, 11: 656.

weighed one or two grams of the massecuite, molasses, etc., which are to be dried and the dish again weighed and the total weight of the matter added, determined by deducting the weight of the dish and cover. About eight cubic centimeters of water are added, the material dissolved with gentle warming, the coil placed in the dish, and the whole dried for two hours. The cover is then replaced and the whole cooled in a desiccator and weighed. The increase in weight represents the dry matter in the sample taken.

The above method of solution of a viscous sample in order to divide it evenly for desiccation is based on the principle of the method first proposed by the author and Broadbent for drying honeys and other viscous liquids.¹⁵ In this process the sample of honey, molasses, or other viscous liquid is weighed in a flat dish dissolved in 80 per cent. alcohol, and then a weighed quantity of pure dry sand added, sufficient to fill the dish three-quarters full. The alcoholic solution of the viscous liquid is evenly distributed throughout the mass and sand by capillary attraction, and thus easily and rapidly dried when placed on the bath.

Pumice stone, on account of its great porosity, is also an excellent medium for the distribution of a viscous liquid in aiding the process of desiccation. The method has been worked out in great detail by Carr and Sanborn, and most excellent results obtained.¹⁶ Round aluminum dishes 2 centimeters high and from 8 to 10 centimeters in diameter are conveniently used for this process. The pumice stone is dried and broken into fragments the size of a pea before use.

28. Method for Unstable Bodies.—For the drying of sirups and other easily altered organic bodies Rolfe and Faxon use a modification of the process proposed by Lobray de Bryn and von Laent.¹⁷ The apparatus is arranged as shown in Fig. 17 so that the greater bulk of the water can be first removed without coming

¹⁵ Chemical News, 1885, 52: 280.

¹⁶ Division of Chemistry, Bulletin 47: 134.

¹⁷ Recueil des Travaux chimiques, 1894, 13: 218.

Journal of the American Chemical Society, 1896, 19: 698.

in contact with the phosphorus pentoxid which is used as the drying agent. This avoids previous evaporation on a water-bath. The tared weighing beaker containing the solution to be evaporated is slipped on the end of the adapter, an air-tight joint being made with "bill-tie" tubing. By the three-way stop-cock, communication can be had with the vacuum pump and with a 250 cubic centimeter flask containing phosphorus pentoxid, or either can be shut off.

The method of drying is as follows: The cock is opened to

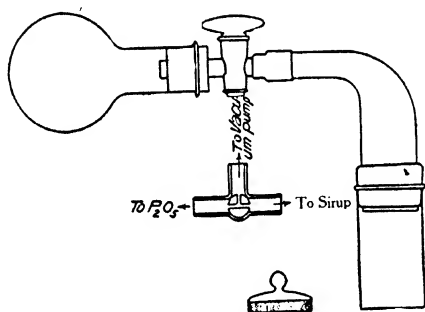


Fig. 17.—Apparatus for Drying Sugars and Sirups.

the pump only, and the air exhausted to 680-690 millimeters. The beaker is lowered into an oil-bath and heated to about 100° till most of the water is evaporated. Communication is then made with the pentoxid flask, and the mass dried to constant weight at about 120° . For convenience, the vapors from the various pieces of apparatus all pass through a four-liter vacuum pan. This large receiver produces an almost instantaneous exhaustion when a stop-cock is opened. Complete drying usually requires from 18 to 24 hours.

29. Danger of Oxidation.—Attention has already been called to the desirability of desiccation in an inert gas. The object of this precaution is to prevent the oxidation of the organic matter some forms of which take up notable quantities of oxygen on long heating. The fats especially are obnoxious to this change. At ordinary temperatures this change does not

so readily take place but even at low temperatures changes from oxidation during long exposure are not unusual. These changes may go on even before all the water in a sample has been expelled—and even a constant weight of two successive periods of drying may be the result of oxidation compensating the further loss of moisture. When, however, an increase of weight is observed after successive periods of drying oxidation may be certainly predicted.

30. Desiccation in Vacuo Without Heat.—To avoid all change in composition due either to heat or oxidation drying in vacuo at room temperatures is practiced. In these cases an exsiccator in which a vacuum is produced by mechanical or chemical means is used. Where the proper pumps are available a very high vacuum is easily secured. The exsiccator is provided with an absorbent usually calcium chlorid or phosphorus pentoxid. Several days and even weeks may be required to remove all the water from the sample and this method is not practicable with samples having over 20 per cent. of water.

31. Production of a Chemical Vacuum.—Vacua are easily produced by removing the air from a closed space with a gas *e.g.*, carbonic dioxid or ether and absorbing the gas in the one case by an alkali and the other by an acid. In an apparatus the size of an exsiccator the latter method is preferable. Benedict and Manning secure an excellent vacuum for practical purposes in a Hempel or Scheibler apparatus as follows:¹⁸

32. Manipulation of Vacuum Desiccator.—The method of producing a high vacuum in a Hempel desiccator depends on the fact that the air in the desiccator is first expelled by ether vapor, and the residual ether vapor is then absorbed by concentrated sulphuric acid. A vacuum of less than one millimeter of mercury can thus be obtained in 10 minutes in a Hempel desiccator containing 2.5 liters of air.

The details of manipulation in connection with the determination of water in samples are as follows:

"The samples (generally two grams) are weighed in aluminum dishes with covers, usually in duplicate. The desiccator

¹⁸ American Journal of Physiology, 1905, 13: 309.

commonly used are of the Hempel form, although the Scheibler desiccator, with the removable acid dish permitting the use of the acid in the upper portion of the desiccator, has been used with equal success.

To economize space and allow the maximum number of dishes in each desiccator, it is advisable to have a rack made with shelves of perforated metal, the shelves to be just far enough apart to permit convenient removal of the aluminum dishes. A brass tube, three millimeters internal diameter, open at both ends, is soldered in the center of the rack extending through the top shelf to just flush with the bottom of the lowest shelf. Such a rack will hold from 16 to 30 aluminum dishes. A ring soldered at a convenient part of the top shelf aids in removing the rack from the desiccator. At one side of the rack it is convenient to fasten the simple manometer which is absolutely essential to the success of the method. As the dishes are placed in the rack, the covers are removed, and for convenience each dish is loosely set in the taper of its cover. The rack is then placed in the perfectly clean, dry desiccator, fresh acid (100-200 cubic centimeters of concentrated, commercial H_2SO_4 , 1.84 specific gravity) placed in the acid compartment, and the whole apparatus carefully connected with a water suction-pump. To prevent "back" suction an empty Dreschel gas washing bottle is placed between the desiccator and the pump, connecting the pump with the tube extending to the bottom of the bottle by means of thick-walled rubber tubing that will not readily collapse. It is well to coat the stopper of the gas washing bottle with a layer of vaseline, or better a mixture of beeswax, Venice turpentine and vaseline. The thicker waxes do not draw into the bottle under greatly diminished pressure as does vaseline. The stop-cock on the desiccator, as well as the edges of the cover, should be well covered with a thin layer of vaseline, or the mixture suggested. The cover should be removed, or slid to one side for a moment, and 10 cubic centimeters of ether delivered from a dry pipette into the upper end of the brass tube extending through the rack. The cover is immediately replaced and the water-suction started. After the exhaustion has commenced the beeswax-vaseline mixture is carefully applied around the edge of the cover. The use of this wax has proven unusually successful in securing a tight closure around the edges of the desiccator and stop-cock. It is easily prepared by melting together 60-75 grams of beeswax, 15 grams Venice turpentine, and 15 grams of vaseline. The proportion of beeswax is varied with

the temperature of the laboratory. In winter, less beeswax will suffice than in summer.

If the delivery tube of the suction-pump dips under water, with an average pump air-bubbles will cease to rise in appreciable quantities in about four or five minutes (with a desiccator containing 2.6 liters). The ether, which can be seen on the bottom of the desiccator, soon begins to boil, and in a few minutes the mercury levels in the manometer begin to approach each other. When the difference in level is about 35 millimeters the stop-cock is closed, the rubber tubing disconnected, and then the suction-pump is stopped. The heavy ether vapor expels all air, and the vapor left in the desiccator when the stop-cock is closed is absorbed by the concentrated sulfuric acid, producing a vacuum of less than one millimeter of mercury in about eight to 10 minutes.

If the stop-cock and cover of the desiccator have been well fitted and well lubricated with wax, the vacuum will hold indefinitely. The desiccator is now placed where it will not be disturbed, preferably in a temperature not above 20°, and left until the materials are dry. The length of time necessary for this operation is in general about two weeks. The manometer should be observed occasionally, and if a leak occurs the desiccator should be exhausted again.

In order to minimize the absorption of moisture from the atmosphere by the dried material on opening the desiccator, we connect the stop-cock of the desiccator, by means of a rubber tube, with the exit tube of a Drechsel gas washing bottle containing concentrated sulfuric acid, and allow the dry air to enter the desiccator through the acid till the pressure becomes atmospheric. A sufficient number of small ordinary desiccators to hold all the aluminum dishes being previously arranged in a convenient place, the cover of the vacuum-desiccator is removed, the rack and dishes withdrawn, the cover of each dish firmly and rapidly pressed into place and the dish placed in one of the smaller desiccators. With a little skill, one can transfer 10 to 20 dishes in this way in a few moments, and no appreciable amount of moisture will be absorbed. The dishes are then immediately weighed. The details of manipulation may seem trifling, but experience has shown that this routine is decidedly advantageous.

33. Drying Experiments With a Vacuum-desiccator.—A number of materials of widely varying nature have been subjected to the drying operation at 100° in air, at 100° in hydrogen, and in high vacua (from 0 to 5 millimeters mercury) over sulphuric

acid. The drying in air and hydrogen at 100° was continued for five hours. The desiccation in vacuo continued for varying lengths of time, as indicated by the varying conditions. As stated before, two grams of material were used in each case, and consequently a variation of 0.1 per cent. in the apparent percentage of water present means a change in weight of but two milligrams.

It is seen that there are marked discrepancies between the percentage of water as determined by heating and by desiccation, and furthermore the percentage as determined by drying in hydrogen is invariably greater than that by drying in air. It is reasonable to suppose from these data that the loss in weight by heat is a resultant of three factors: first, loss in weight of water; second, volatilization of material other than water; third, oxidation. To these three factors may be added the possibility of the actual splitting off of water from the protein or carbohydrate molecule, such as, for example, the formation of an anhydride.

A number of experiments were made on materials of vegetable and animal nature to observe the effect of prolonged drying in a high vacuum over sulfuric acid. Comparative tests with heat were not made.

The materials of animal origin included a number of albuminoids which were under investigation at the same time in this laboratory.

With these animal materials it is seen that, in general, the drying operation is complete at the end of two weeks, and subsequent desiccation is without material effect on the absolute moisture-content. This is nowhere more markedly shown than in the case of the albuminoids that were kept in a high vacuum for six months. The subsequent loss in weight after the first two weeks was inconsiderable in all cases save one. It is to be regretted that weighings were not made at the end of three weeks with this set of samples, for probably the major portion of the slight loss took place during the third week.

The complete dehydration of butter in a high vacuum is of especial interest, when we consider the difficulties usually experienced in securing an equal distribution of the material in the sample dish and the passage of the water through the supernatant layer of melted fat. It is worthy of note that in several tests made with the vacuum method no especial degree of importance could be ascribed to an even distribution of the sample. Desiccation was equally complete whether the sample was first

melted in a thin layer on the bottom of the dish, or large irregular lumps of butter were used.

With vegetable materials we find a continued slight loss for several weeks, and while the loss in weight during the first two weeks is probably as close a measure of the absolute amount of water present as is the loss in weight after heating at 100° for five hours, nevertheless, the continued slight loss in weight in a vacuum signifies that moisture is still present.

The cereal products, bread and shredded wheat, show this continued loss most markedly, while singularly enough a sample of gingersnaps does not exhibit the water-retaining property of the other two.

Fæces were included under the head of animal materials, since, in all probability, according to Prausnitz, the fæces consist more of metabolic products, *i. e.*, biliary residues, intestinal debris, etc., than of undigested vegetable material. As a matter of fact, the sample of fæces here used was that from a man living essentially on a vegetable diet. It is obvious that the large differences between the per cent. of water as found by the two methods with heat and the third method by vacuum is attributable to the volatilization of large amounts of material other than water.

The length of time required to secure complete desiccation in a vacuum is perhaps the most interesting feature of these results. Obviously, a marked difference exists between materials of a vegetable and those of an animal nature, the former retaining their moisture persistently while the latter have, in general, lost all but mere traces of their moisture after two weeks in a high vacuum. For animal materials, therefore, we may say that two weeks in a high vacuum suffices to remove practically all moisture. For the most accurate work a longer time is to be recommended, though the slight loss in weight subsequent to two weeks' desiccation can hardly affect ordinary physiological or chemical research.

With vegetable materials, desiccation should proceed for a period longer than two weeks. As a matter of fact, however, it can readily be seen that the sample, as soon as prepared for analysis, can be placed in the vacuum and weighed as soon after two weeks as the results needed. In general, the time between the preparation of a sample for analysis, and the time when the results are imperatively needed, is considerably over two weeks. For preliminary determinations in metabolism experiments, where an approximate knowledge of the water-content of foods is necessary, the usual five-hour heating will suffice, and

the final calculations may be based on the determination by the vacuum method. For technical work, obviously the method is, as already stated, too time-consuming, though the advantages may appeal to the technical analyst and the method may possibly find some use in special cases.

34. Experiments on the Absorption of Water by Desiccated Material.—The marked difference in time required to remove moisture completely from animal and vegetable material suggested the idea that in materials of a vegetable origin the structure of the cell-walls might be such as to retard considerably the evaporation of water. Were this the case, we should expect that after the samples had once become thoroughly dry, moisture subsequently absorbed would be retained in a different manner, and in all probability more easily given up in a high vacuum. Samples of beef, bread, faeces, gingersnaps, and shredded wheat were removed from the high vacuum in which they had been placed several weeks before. After weighing, they were exposed with the covers off to air saturated with water vapor at 30° for two and one-half hours. The gain in weight was marked in all cases. The samples were then placed immediately in a high vacuum, and weighed at the end of 18, 40, and 112 hours, respectively.

The data indicated that much more water was absorbed than was originally in sample.

35. General Principles of Drying Samples.—It would be a needless waste of space to go into further details of devices for desiccation. A sufficient number has been given to fully illustrate all the principles involved. In general, it may be said that drying in the open air at a temperature not exceeding that of boiling water can be safely practiced with the majority of samples. For instance, we have found practically no change of importance in the composition of cereals dried in the air and in an inert gas. The desiccation should in all cases be accomplished as speedily as possible. To this end the atmosphere in contact with the sample should be dry and kept in motion. An oven surrounded by boiling water and steam is to be preferred to one heated by a flame. Constancy of temperature is quite as important as its degree and this steadiness is most easily secured by steam at atmospheric pressure. Where higher temperatures than 100° are desired the steam must be under pressure, or the boiling-point of the water may be raised by adding salt or other

soluble matters. A bath of paraffin or calcium chlorid may also be used or a sand or air-bath may be employed. The analyst must not forget, however, that inorganic matters are prone to change at temperatures above 100° , even in an inert atmosphere, and higher temperatures must be used with extreme caution.

Drying in partial vacuum and in a slowly changing atmosphere may be practiced with all bodies and must be employed with some. The simple forms of apparatus already described will be found useful for this purpose. At a vacuum of 20 inches or more, even unstable organic agricultural products are in little danger of oxidation. In the introduction of a dry gas, therefore, air will be found as a rule entirely satisfactory. In the smaller forms of vacuum apparatus described, however, there is no objection to the employment of hydrogen or of carbon dioxid. The gas entering the apparatus should be dried by passing over calcium chlorid or by bubbling through sulfuric acid. The vacuum may be provided by an air-pump connected with a large exhaust cylinder. This cylinder is connected by a system of pipes to all the working desks. The chief objection to this system is the unsteadiness of the pressure. When only a few using the vacuum apparatus for filtering or other purposes the vacuum will stand at about 20 inches. When no one is using it the vacuum will rise to 28 or 29 inches. At other times, when in general use, it may fall to 15 inches. Where a constant vacuum is desired for drying, therefore, it is advisable to connect the apparatus with a special aspirator which will give a pressure practically constant. A large vacuum pump such as is found on locomotives for working the air-brakes, attached to a large air-tight cylinder will give very satisfactory service in a large laboratory. Each of the working desks is connected with the vacuum cylinder.

The dishes containing the sample should be low and flat, exposing as large a surface as possible. For viscous liquids it will be found advisable to previously fill the dishes with pumice stone or other inert absorbent material to increase the surface exposed.

The special methods of drying milk, sirup, honeys, and like bodies, will be described in the paragraphs devoted to these substances.

In drying agricultural products, not only water but all other matters volatile at the temperature employed are expelled. It is only necessary to conduct the products of volatilization through sulfuric acid to demonstrate the fact that organic bodies are given off. In the cases mentioned the sulfuric acid will be speedily changed to a brown and even black color by these bodies. It is uncontestable, however, that in most cases the essential oils and other volatile matters thus escaping are not large in quantity and could not appreciably affect the percentage composition of the sample. In such cases the whole of the loss on drying is entered in the note-book as water. There are evidently many products, however, where a considerable percentage of the volatile products is not water. The percentage of essential oils, which have a lower boiling-point than water, can be determined in a separate sample and this deducted from the total loss on drying will give the water.

Simple as it seems, the determination of water in agricultural products often presents peculiar difficulties and taxes to the utmost the patience and skill of the analyst. Having set forth the substantial principles of the process and indicated its more important methods, there is left for the worker in the laboratory the choice of processes already described, or, in special cases, the device of new ones and adaptation of old ones to meet the exigencies which may arise.

INCINERATION

36. Definition of Ash.—The term ash is variously interpreted by analysts. Its most obvious meaning includes the incombustible residue of burning a body in the air. For chemical purposes, however, the temperature of the incineration, the shape of the vessel in which it is conducted and the kind of furnace in which it takes place are all matters of interest. All of these factors influence the quantity as well as the composition of the residue. The true ash comes only from the material burned. Any min-

eral substance, however, which may be found with the body will appear as a part of the ash. Also any unburned carbon will be included. In addition to this, carbon dioxid produced during the combustion, takes the place of organic acids previously combined with the bases. The whole residue is called crude ash and when treated with an acid and filtered and thus freed of carbon, carbon dioxid, and sand, it is "pure ash."

37. Determination of Ash.—The principle to be kept in view in the preparation of the ash of agricultural products is to conduct the incineration at as low a temperature as possible to secure a complete combustion. The danger of too high a temperature is twofold. In the first place some of the mineral constituents constantly present in the ash, notably, some of the salts of potassium and sodium are volatile at high temperatures and thus escape detection. In the second place, some parts of the ash are rather easily fusible and in the melted state occlude particles of unburned organic matter, and thus protect them from complete oxidation. Both of these dangers are avoided, and an ash practically free of carbon obtained, by conducting the combustion at the lowest possible temperature capable of securing the oxidation of the carbonaceous matter.

38. Products of Combustion.—The most important product of combustion, from the present point of view, is the mineral residue obtained. The organic matter of the sample undergoes decomposition in various ways, depending chiefly on its nature. Complex volatile compounds are formed first largely of combustible matter. The residual carbon is oxidized to carbon dioxid and the hydrogen to water. The relative proportions of these bodies formed, in any given case, depend on the conditions of combustion. With a low temperature and a slow supply of oxygen, the proportion of volatile organic compounds is increased. At a high temperature, and in a surplus of oxygen, the proportions of water and carbon dioxid are greater. At the present time, however, our attention is to be directed exclusively to the mineral residue; the organic products of combustion belonging to the domain of organic chemistry. As has already been intimated, the ash of agricultural samples consists of the mineral matters

derived from the tissues, together with any accidental mineral impurities which may be present, some unburned carbon, and the sulfur, phosphorus, chlorine, nitrogen, etc., existing previously in combination with the mineral bases. The organic sulfur and phosphorus may also undergo complete or partial oxidation during incineration and be found in the ash. Unless special precautions be taken, however, a large portion of the organic sulfur and phosphorus escapes as volatile compounds during the combustion.¹⁹ Such volatile compounds cannot be regarded as constituents of normal ash. The organic nitrogen is probably completely lost, at most, only traces of it being oxidized during the combustion in such a way as to combine with a mineral base. The rare mineral elements that are taken up by plants will also be found in the ash. Here the analyst would look for copper, boron, zinc, manganese, and the other elements which, when existing in the soil, are apt to be found in the tissues of the plants, not, perhaps, as organic or essential compounds, but as concomitants of the other mineral foods absorbed by growing vegetation. This fact is often of importance in toxicological and hygienic examinations of foods. For instance, traces of copper or of boron in the ash of a proscribed food would not be convincing evidence of the addition of copper or borax salts unless it could be shown that the soil on which the food in question was grown was free of these bodies.

This fact manifestly applies only to those cases where mere traces of these rare bodies are in question. The presence of considerable quantities of them, enough to be inimical to health, could only be attributed to artificial means.

39. Purpose and Conduct of Incineration.—In burning a sample of an agricultural product the analyst may desire to secure either a large sample of ash for analytical purposes as already described or to determine the actual percentage of ash. The first purpose is secured in many ways. In the preparation of ash for manurial purposes, for instance, little care is exercised either to prevent volatilization of mineral matters or to avoid the occurrence of a considerable quantity of carbon in the sample. With this

¹⁹ Volume I, Second Revised Edition: 460, 470.

operation we have, at present, nothing whatever to do. In preparing a sample of ash for chemical analysis it is important, where a sufficient quantity of the sample can be obtained, to use as large a quantity of it as convenient. While it is true that very good results may be secured on very small samples, it is always advisable to have good supply of the material at hand. Since the materials burned have usually only from one to three per cent. of ash, a kilogram of them will supply from 10 to 30 grams. To supply all needful quantities of material and replace the losses due to accident, whenever possible at least 20 grams of the ash should be prepared. The combustion can be carried on in platinum dishes with all bodies free of metallic oxids capable of injuring the platinum. Otherwise porcelain or clay dishes may be employed. As a rule the combustion is best conducted in a muffle at a low red heat. With substances very rich in fusible ash, as for instance the cereals, it is advisable to first char them, extract the greater part of the ash with water, and afterwards burn the residual carbon. The aqueous extract can then be added to the residue of combustion and evaporated to dryness at the temperature of boiling water. During the combustion the contents of the dish should not be disturbed until the carbon is as completely burned out as possible. The naturally porous condition in which the mass is left during the burning is best suited to the entire oxidation of the carbon. At the end, however, it may become necessary to bring the superficial particles of unburned carbon into direct contact with the bottom of the dish by stirring its contents. In most instances very good results may be obtained by burning the ash in an open dish without the aid of a muffle. In this case a lamp should be used with a spreading flame covering as evenly as possible the bottom of the dish and thus securing a uniform temperature. The carbon, when once in active combustion, will as a rule be automatically consumed, and an ash reasonably pure be obtained.

The second purpose held in view by the analyst is to determine the actual content of ash in a sample. For this purpose only a small quantity of the material should be used, generally from two to 10 grams. The combustion should be conducted

in flat-bottomed, shallow dishes, and at a low temperature. In many cases the residue, after determining the moisture, can be at once subjected to incineration, and thus an important saving of time be secured. A muffle, with gentle draft, will be found most useful for securing a white ash. The term, white ash, is sometimes a deceptive one. In samples containing iron or manganese, the ash may be practically free of carbon and yet be highly colored. The point at which the combustion is to be considered as finished, therefore, should be at the time the carbon has disappeared rather than when no coloration exists. In general the methods of incineration are the same for all substances, but some cases may arise in which special processes must be employed. Some analysts prefer to saturate the substance before incineration with sulfuric acid, securing thus a sulfated ash. This is practiced especially with molasses. In such cases the ash obtained is free of carbon dioxid and roughly the difference in weight is compensated for by deducting one-tenth of the weight of the ash when comparison is to be made with ordinary carbonated ash. Naturally this process could not be used when sulfuric acid is to be determined in the product.

40. German Ash Method.—The method pursued at the Halle Station for securing the percentage of ash in a sample is as follows:²⁰ Five grams of the air-dried sample are incinerated in a platinum dish and the ash ignited until it has assumed a white, or at least a light gray tint. As soon as combustible gases are emitted at the beginning of the incineration they are ignited and allowed to burn as long as possible. It is advisable to hasten the oxidation by stirring the mass with a piece of platinum wire. If the ash should become agglomerated, as sometimes happens with rich food materials, it must be separated by attrition. The ash, when cooled in a desiccator, is to be weighed. When great exactness is required, it is advised, as set forth in a former paragraph, to first carbonize the mass and then extract the soluble ash with hot water before completing the oxidation. When

²⁰ Bieler and Schneidewind, *Die agricultur-chemische Versuchs-Station*, Halle a/S:34.

the latter is complete and the dish cooled the aqueous extract is added, evaporated to dryness and the incineration completed.

41. Injury to Platinum Dishes.—When platinum dishes are used in determining ash special precautions must be observed to avoid injury to the metal. The ash of cereals is apt to be very acid on account of an excessive quantity of phosphoric acid. In other words the acid phosphates predominate over the neutral. It is difficult to burn finely divided carbon in contact with acid phosphates without a reduction of a part of the phosphorus. In these cases the reduced phosphorus is very apt to work serious injury to the platinum dish.

42. Courtonne's Muffle.—The ordinary arrangement of a

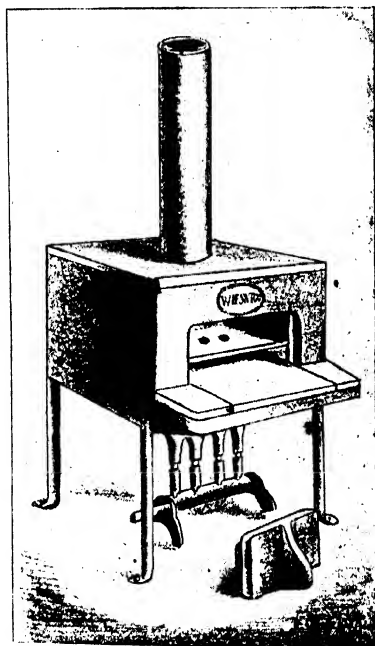


Fig. 18. Courtonne Muffle.

muffle, as in assaying, may be conveniently used in incineration.

A special muffle arrangement has been prepared by Courtonne which not only permits of the burning of a large number of samples at once, but also effects a considerable saving in gas.²¹ The muffle as shown in Fig. 18, is made in two stages, and the floor projects in front of the furnace, forming a convenient hearth. The incineration is commenced on the upper stage, where the temperature is low, and finished on the lower one at a higher heat. The furnace is so arranged as to permit the flame of the burning gas to entirely surround the muffle. The draft and temperature within the muffle are controlled by the fire-clay door shown resting on the table.

43. Combustion Furnace for Ashing.—Schneider has called attention to a simple modification of an ordinary combustion furnace which adapts this apparatus to the determination of ash.²² By simply turning the fire-clay sides of the combustion furnace very good results are obtained.

44. Resume of Standard Methods for Determining the Ash of Plants.—A valuable study of the methods of ashing plants has been made by Tollens.²³

In order to regulate the admission of air to the substance and at the same time to retain the volatile substances, several forms of apparatus have been proposed in which the substance is burned in a current of air or oxygen, and the volatile portion or fine particles collected. König used simply a glass tube drawn out to a point for discharging a small current of oxygen into the faintly glowing substance.

Mitscherlich²⁴ and Schloesing²⁵ heated the substances in a platinum or silver boat placed in a wide glass or porcelain tube, through which a current of carbon dioxide was first passed, until the charring was complete and combustible gases ceased to be given off, and then a current of oxygen, and the incineration completed. By this means there should be hardly any volatilization of substance and no inclosing of carbon by the fusing of the salts. A similar apparatus has been recommended by Reese.²⁶

²¹ Sidersky, *Traité d'Analyse des Matières Sucrées*: 176.

²² *Chemiker-Zeitung*, 1904, 17: 78.

²³ *Experiment Station Record*, 1901-2, 13: 207, 305.

²⁴ *Journal für praktische Chemie*, 1845, 36: 232.

²⁵ *Encyclopédie chimique*, 1885, 10: 225.

²⁶ *Zeitschrift für analytische Chemie*, 1888, 27: 133.

Hlasiwetz²⁷ employed in a double necked flask, in one opening of which a porcelain tube was placed in a perpendicular position, wide above and narrow below, with a sieve of platinum in the bottom upon which the substance was placed. The substance was ignited and air was drawn through the tube and the bottle by means of an aspirator until the combustion was complete. The products of combustion were passed through the water in the flask, which held any particles carried along mechanically, as alkaline chlorids, etc. The incineration was completed in a platinum dish.

Shuttleworth²⁸ and Tucker,²⁹ working in the Agricultural-Chemical Laboratory of the University of Göttingen, have recently made use of another principle. They employed a specially constructed platinum crucible of large size, with a platinum cover carrying a tube reaching to the bottom of the crucible, through which a current of air was conducted, and with an arrangement for retaining any particles of volatilized substance. The construction of the two forms of apparatus is shown in the accompanying figures 19 and 20.

A weighed amount of the substance is placed in the crucible without the cover, and charred on a sand bath until no more gases are given off. The cover with the tube for conducting air is then put in place and the crucible heated over a naked flame. The air is forced into the Shuttleworth apparatus (Fig. 19), while in the Tucker apparatus (Fig. 20) it is drawn through by means of an aspirator, the tube conducting the current of air to the bottom of the crucible carrying a stirrer in both cases. In the Shuttleworth apparatus the loss of light particles of matter is avoided by an attachment to the cover (*d'*, *b*,) containing water (*c*). In the Tucker apparatus the air drawn off is passed through a small flask (*g*) containing water, in which the volatilized chlorids, etc., are collected.

During the combustion the substance is stirred by a platinum stirrer, which in the Shuttleworth apparatus is attached to the tube introducing air into the crucible, and in the Tucker apparatus extends down through the inlet tube.

The Tucker apparatus has the advantage over the Shuttleworth apparatus of simplicity of construction and ease of operation. Again, the cover of the Shuttleworth apparatus is complicated, and forcing the air through is less satisfactory than

²⁷ *Annalen der Chemie*, 1856, **97**: 244.

²⁸ *Journal für Landwirtschaft*, 1899, **47**: 173.

²⁹ *Journal für Landwirtschaft*, 1900, **48**: 64.

drawing it through, as in Tucker's apparatus. The conical form of the crucible of Tucker's apparatus is also preferable, permitting the heat to be applied more uniformly.

With this apparatus, straw, leaves, and potatoes are incin-

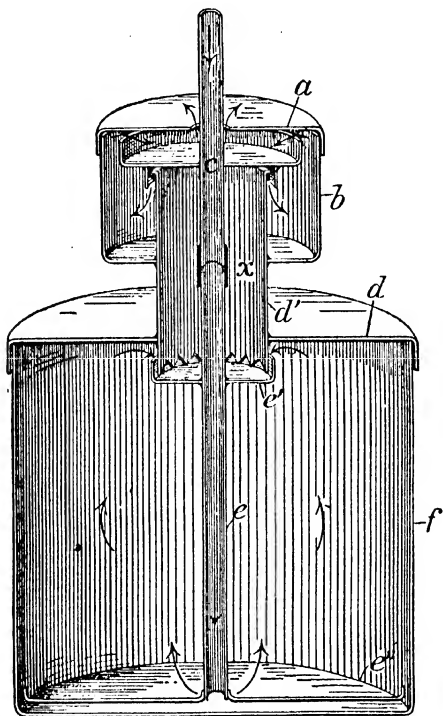


Fig. 19.—Shuttleworth Apparatus for Ash Determination.

erated in the course of two hours, the charring occupying about $1\frac{1}{2}$ hours, and the completion of the incineration in a current of air requiring not more than a half hour.

H. Wislicenus³⁰ has recently described an apparatus which embodies the principle of the Tucker apparatus, but consists only of a platinum cover which may be adapted to dishes and crucibles ordinarily found in laboratories, thus doing away with the special crucible.

³⁰ Zeitschrift für analytische Chemie, 1901, 40 : 441.

Another form of incinerating apparatus is the Berthelot bomb, in which the substance is burned, by means of an electrically heated wire, in oxygen under 25 atmospheres pressure. This apparatus has the advantage of excluding every possible loss, and is absolutely closed during the operation; but the manipulation of it is somewhat difficult, special care being necessary in collecting the products of combustion, and, as Berthe-

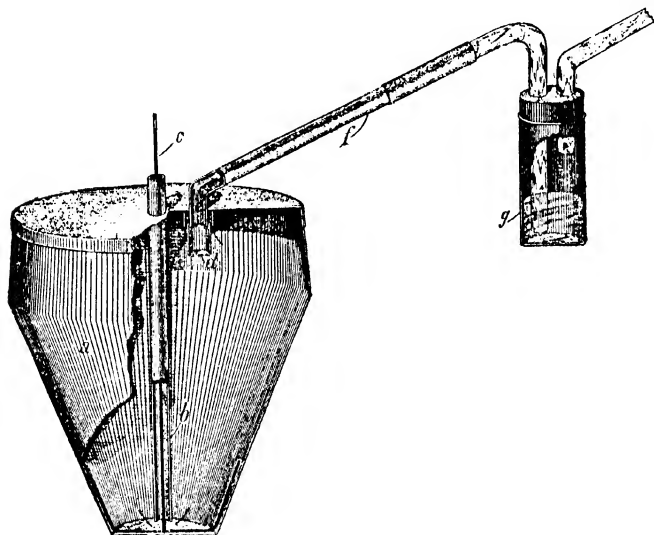


Fig. 20. Tucker Apparatus for Ash Determination.

lot states, it burns only small amounts at a time.³¹ Furthermore, the apparatus is quite expensive, and necessitates a supply of compressed oxygen. The same applies to the modified apparatus constructed by Mahler and by von Hempel and Atwater.³²

Grouven³³ destroyed organic matter by combustion in superheated steam in iron tubes. In this method the sulfur occurring in plants in such compounds as protein etc. is not oxidized to sulfuric acid, as in the usual process of burning, but is obtained as sulfuretted hydrogen.

It sometimes happens that the incineration occupies a long time even in the Shuttleworth and Tucker forms of apparatus ex-

³¹ *Annales de Chemie et de Physique*, 1885, 6 : 546.

³² *Office of Experiment Stations, Bulletin* 21, 1895 : 123.

³³ *Die landwirtschaftlichen Versuchs-Stationen*, 1883, 28 : 343.

ceeding the two hours which is usually sufficient; for example, when large quantities of fusible salts and especially alkaline phosphates are present. In such cases the carbon particles are occluded from the action of oxygen of the air; but in burning in an open platinum dish this occurs to a much greater extent, so that the incineration may sometimes require 10 to 12 hours and even longer. In such cases the danger from loss of alkaline salts is naturally great, increasing as the temperature is raised.

In order to prevent the enveloping of particles of carbon due to the fusing of salts, these salts may be removed prior to the combustion. In the method of Fresenius,³⁴ frequently used, the charred substance is extracted with water or acetic acid,³⁵ the insoluble portion collected on a filter, dried and incinerated and the soluble portion then added, evaporated, and gently incinerated.³⁶ This operation requires both time and pains.

The fusing of the salts may also result in the formation of silicates which are difficult to decompose with hydrochloric acid, as in the case of straw which contains much silicic acid, and this is an important point to be observed, as Shuttleworth has pointed out.³⁷ In such case the portion of the ash insoluble in hydrochloric acid must be treated with hydrofluoric acid, and the bases contained in that portion estimated separately.

ADMIXTURES TO FACILITATE INCINERATION

To overcome the difficulties of incineration, attempts have been made for many years to discover some substance to be added to the mass which would prevent the fusing of the salts and unite with the volatile substances, like chlorin, sulfuric acid, and phosphoric acid, so as to avoid their loss.

As an oxygen-furnishing material and to facilitate the incineration Verdeil³⁸ employed ammonium nitrate, and Keller nitric acid, for animal substances. Platinum sponge was first employed by H. Rose.³⁹ This material, however, is somewhat expensive. Platinum chlorid, employed by Rose and Fleitman,⁴⁰ is similar in its action, but naturally the volatilization of an indeterminate amount of chlorin from the platinum chlorid ren-

³⁴ *Journal für praktische Chemie*, 1857, **70** : 85.

³⁵ *Städeler in Wöhler's Mineralanalyse in Beispielen*, 2d Edition, 1861 : 188.

³⁶ *Archiv der Pharmacie*, 1853, **73** : 258.

³⁷ *Journal für Landwirtschaft*, 1899, **47** : 173.

³⁸ *Liebigs Annalen der Chemie*, 1849, **69** : 89.

³⁹ *Annalen der Physik und Chemie*, 1850, **80** : 101.

⁴⁰ *Jahresbericht über die Fortschritte der Chemie*, 1849 : 595.

ders it impossible to accurately determine the chlorin in the ash. Admixtures of iron oxid, as recommended by Gräger,⁴¹ and of ferric nitrate, employed by A. Müller,⁴² likewise accelerate the combustion and may be useful in some cases. In employing mercuric oxid, as practiced by Will, the analyst must guard against the mercury vapor given off. The addition of sand likewise facilitates the burning, and Alberti and Hempel⁴³ recommend in incinerating sugar products, molasses, etc., adding a weighed amount of quartz sand, which is afterwards subtracted from the total weight of the ash. However, by the use of sand chlorin, sulfuric acid, and phosphoric acid are at least partially driven off.

Kassner⁴⁴ mixed calcium plumbate (Ca_2PbO_4) with the substance, thereby facilitating the burning, but interfering with the later analysis of the ash. The same objection applies to bismuth nitrate, which Béchamp⁴⁵ recommended. Among other admixtures suggested may be mentioned pumice stone, copper oxid, clay, and magnesia, employed by Donath.⁴⁶ According to Donath, these are inferior (at least with sugar products) to incineration at first in dilute and latter in pure oxygen. Recently H. Wislicenus⁴⁷ has suggested moistening the greyish ash with pure hydrogen peroxid which facilitates the complete combustion of the carbon.

Most frequently, however, admixtures of an alkaline character are employed, and these, while facilitating the combustion, are also of real value in preventing the volatilization of chlorin and sulfuric acid, which otherwise are in danger of being lost. Strecker⁴⁸ has employed baryta, Way and Ogstone⁴⁹ barium nitrate, Slater⁵⁰ barium superoxid, and Wackenroder⁵¹ lime, calcium carbonate, and calcium acetate, all with good results. Von

⁴¹ Liebig's Annalen der Chemie, 1859, **111** : 124.

⁴² Journal für praktische Chemie, 1860, **80** : 118.

⁴³ Zeitschrift des Vereins für die Rübenzucker-Industrie des Deutschen Reichs, 1891, **41** : 743.

⁴⁴ Zeitschrift für analytische Chemie, 1891, **30** : 44.

Archiv der Pharmacie, 1890, **228** : 171.

⁴⁵ Comptes rendus, 1871, **73** : 337.

⁴⁶ Zeitschrift des Vereins für die Rübenzucker-Industrie des Deutschen Reichs, 1891, **41** : 740.

⁴⁷ Zeitschrift für analytische Chemie, 1901, **40** : 443.

⁴⁸ Liebig's Annalen der Chemie, 1850, **78** : 346.

⁴⁹ Jahresbericht über die Fortschritte der Chemie, 1849 : 600.

Journal of the Royal Agricultural Society of England, 1847, **8** : 134.

⁵⁰ The Chemical Gazette, 1855, **13** : 53.

⁵¹ Archiv der Pharmacie, 1848, **53** : 1.

Schröder and Reuss⁵² in the analysis of forest products impregnated the substance with a solution of sodium carbonate previous to charring, to prevent the loss of chlorin and sulfuric acid. Counciler⁵³ added to each gram of substance to be burned one cubic centimeter of a 10 per cent. soda solution, dried, charred, and incinerated over an alcohol lamp., Behaghel von Adikerskron and Bunge⁵⁴ earlier showed that in incinerating animal substances correct figures for the chlorin content were obtained only with the addition of sodium carbonate.

Shuttleworth⁵⁵ found the addition of a measured quantity of calcium acetate of known calcium content very advantageous, the lime resulting from the higher heating retaining the chlorin and preventing the fusing together of the salts, so that the mass remained porous and difficultly decomposed silicates were not formed. This admixture is especially to be recommended in case of substances like straw, which are rich in silicic acid, with leaves, and with seeds rich in phosphoric acid. With potatoes it is not necessary as von Daszewski⁵⁶ has found, because they are easily and completely incinerated without such addition.

A further advantage of the addition of alkaline substances in the incineration is the avoidance of the formation of pyro-phosphate in the ash and incomplete precipitation of the phosphoric acid, which may result in case of insufficient treatment of the ash solution with nitric acid.⁵⁷

Addition of sulfuric acid in incineration.—In the analysis of molasses and other sugar products, the incineration is now generally carried on with the aid of sulfuric acid, as proposed by Scheibler.⁵⁸ This addition simplifies the operation very materially, but the bases are naturally all recovered in the ash as sulfates. As this increases the weight over the bases themselves or their carbonates, a correction must be made in the weight of the sulfate ash. Although this factor is now commonly used for the correction, it is only a conventional factor and by no means accurate under all circumstances. Hence, others as

⁵² Die Beschädigung der Vegetation durch Rauch, 1883: 131.

⁵³ Landwirtschaftlichen Versuchs-Stationen, 1882, 27: 375.

⁵⁴ Zeitschrift für analytische Chemie, 1873, 12: 390.

⁵⁵ Journal für Landwirtschaft, 1899, 47: 173.

⁵⁶ Journal für Landwirtschaft, 1900, 48: 223.

⁵⁷ Von Raumer, Zeitschrift für analytische Chemie, 1881, 20: 376.

⁵⁸ Zeitschrift des Vereins für die Rübenzucker-Industrie im Zollverein, 1864, 14: 188; 1867, 17: 338.

Biard,⁵⁹ Sidersky, von Lippmann and Wiechmann,⁶⁰ have preferred to deduct one-fifth of the ash; and, because of this uncertainty, Alberti and Hempel advocate their method of combustion with a known amount of quartz sand, as mentioned above. Hehner⁶¹ has also proposed the addition of sulfuric acid in the combustion of glycerine, using the factor 0.8.

It is apparent that ash prepared with the addition of sulfuric acid can not be employed for the later estimation of that acid, or for chlorine and probably not for phosphoric acid.

45. Methods of the Official Agricultural Chemists.—The methods of ash determination adopted by the Association of Official Agricultural Chemists vary with the purpose in view.⁶² Experience has shown that the peroxid of sodium method for the determination of sulfur is also applicable to the determination of organic phosphorus. The methods of ash analysis are fully described in the volume referred to above.

46. Special Determination of Sulfur.—Both organic sulfur and phosphorus are likely to escape oxidation during ordinary combustion unless special precautions to insure complete oxidation are employed. The official method of securing all the sulfur presents many difficulties of manipulation which render desirable a simpler and shorter process. Schreiber has devised a method which presents some distinct advantages in these respects.⁶³

One gram of material is placed in a nickel crucible of 100 cubic centimeters capacity, with 10 cubic centimeters of a solution made by dissolving 100 grams of sodium nitrate and 150 grams of sodium hydrate in 500 cubic centimeters of water. Add five grams of crystallized magnesium nitrate and stir with a platinum rod, making sure that the mass is thoroughly mixed and that the sample is broken up as much as possible. Wash down the material adhering to the stirring rod and sides of the crucible with the smallest possible amount of water. Heat for one hour on a hot-plate covered with a thin sheet of asbestos

⁵⁹ *Neue Zeitschrift für Rübenzucker-Industrie*, 1889, **23**: 165.

⁶⁰ *Zeitschrift des Vereins für die Rübenzucker-Industrie des Deutschen Reichs*, 1891, **41**: 93.

⁶¹ *Journal of the Society of Chemical Industry*, 1889, **8**: 4.

⁶² Bureau of Chemistry, Bulletin 107 Revised: 21.

Principles and Practice of Agricultural Analysis, 2nd Revised Edition, 1908, **2**: 617-621.

⁶³ Bureau of Chemistry, Circular No. 56, 1910.

paper, keeping the temperature at about 130° . Put the cover on the crucible, tilting it in such a fashion as to leave an opening for the steam to escape and heat further for one hour at from 150° to 160° or until the material is entirely dry. If the fusions begin to bump, lower the heat so that the covers will not be jarred down tight on the crucibles, and the material lost by frothing. When the mass is entirely dry, put the covers on tight and heat gradually until the temperature reaches 180° , then heat for 35 minutes, maintaining the temperature at about 180° to 200° .

Set the crucible (with the cover on tight) into a round hole in a piece of asbestos board, so that about 1.5 inches of the lower part of the crucible shall project below the asbestos board. Heat with the bunsen burner for half an hour, allowing the flame to just touch the bottom of the crucible during the first 15 minutes, and then with the full heat during the last 15 minutes. During the first five minutes of heating with the full flame keep the crucible in an upright position, then remove the cover and tilt the crucible so as to fuse any material which may have crept up the sides. Then return the crucible to the upright position, replace the cover, and heat for five minutes more. When the fusion has solidified, and before it has entirely cooled, place the crucible in a 600 cubic centimeter beaker with 150 cubic centimeters of distilled water and cover with a watch-glass. Put the crucible cover in the beaker and slightly rotate and tilt the beaker so that all parts of the crucible shall be touched by the water. Run in 13 cubic centimeters of hydrochloric acid (specific gravity 1.19) from a burette and again rotate the beaker slightly. Tip the crucible so that the other side comes in contact with the acid liquor, allow it to stand a few minutes, and remove the crucible and cover, washing the liquid adhering to them back into the beaker by means of distilled water. Any fused material adhering to the sides of the crucible can easily be removed with a stirring rod. Place the beaker on the steam-bath and heat for about half an hour and then let it stand in the cold over night. Filter and wash the insoluble residue. Heat the filtrate on the steam-bath or otherwise, and precipitate with a 10 per cent. barium chlorid solution.

In this method a crucible loses from 0.3 to 1.3 grams of its weight in each determination, an average of 0.9 gram as calculated from 20 determinations. By the peroxid method the crucible loses from 1.2 to two grams of its weight in a fusion, an average of 1.4 grams based on 13 determinations. Using a griddle hot-plate, 14 by 18 inches, 18 fusions can easily be made

in a day. The only way in which a determination can be lost is by heating too rapidly on the hot-plate, thus causing it to boil over or spatter.

Skinner comparing the Barlow-Tollens and peroxid methods found that the latter gave slightly higher results in five out of seven cases, the difference averaging about $+0.11$ per cent. of SO_4 or $+0.036$ per cent. of sulfur.⁶¹ The proposed method gives sulfur results about 0.1 per cent. lower than the peroxid method, the average of the 12 values which were lower by the proposed method would thus be about 0.064 per cent. less than by the absolute method. Both of these discrepancies are well within the experimental error. Also it should be noted that the difference between the figures obtained by fusing the barium sulfate in the peroxid method and reprecipitating, and those resulting from the first precipitation by the proposed method vary from -0.04 to $+0.11$ per cent. In six out of 12 cases the figures obtained by the first precipitation in the proposed method are nearer the values obtained by fusing and reprecipitating the barium sulfate in the peroxid method than they are to the results of the first precipitation in the peroxid method. The peroxid method gives a very high blank as compared with the proposed method; with the former this blank amounted to from 0.0009 to 0.0028 per cent. of sulfur and in the latter it amounts to from 0 to 0.0007 per cent. As the blanks by the peroxid method are very high an error would be introduced if they were not run in each set.

The values obtained by these different methods rarely approached those by the peroxid method nearer than 0.1 per cent. which suggests that the latter may be too high. It was therefore, decided to examine a new lot of samples by the peroxid method, fuse the barium sulfate obtained with soda, reprecipitate, and determine the sulfur by the proposed method.

It appears that the first precipitation of the sulfuric acid by this method gives values very nearly approaching those obtained by fusing the barium sulfate obtained by the peroxid method and reprecipitating. The differences between the two sets of results thus obtained range from -0.04 to $+0.11$, per cent (an average of 0.04 per cent); these differences are within the experimental error.

TREATMENT WITH SOLVENTS

47. Object of Treatment.—The next step, in the analytical work, after sampling, drying, and incinerating, is the treatment

⁶¹ Bureau of Chemistry, Bulletin 116:92.

of the sample with solvents. The object of this work is to separate the material under examination into distinct classes of bodies distinguished from each other by their solubilities. It is not the purpose of this section to describe the various bodies which may be separated in this way, especially from vegetable products. For this description the reader may consult the standard works on plant analysis.⁶⁵

The chief object of a strictly agricultural examination of a field or garden product is to determine what has been taken from the soil and its food value. This purpose can be accomplished without entering into a minute separation of nearly allied bodies. For example, in the case of carbohydrates it will be sufficient as a rule, to separate them into four classes. In the first class will be found those soluble in water as the ordinary sugars. In the second group will be found those which, while not easily soluble in water, are readily rendered so by treatment with certain ferments or by hydrolysis with an acid. The starches are types of this class. In the third place are found those bodies which resist the usual processes of hydrolysis either with an acid or alkali, and therefore remain in the residue as fiber. Cellulose is a type of these bodies. In the fourth class are included those bodies which on hydrolysis with an acid yield furfural on distillation, and therefore belong to the type containing five atoms of carbon or some multiple thereof in their molecule. For ordinary agricultural purposes the separation is not even as complete as is represented above.

What is true of the carbohydrates applies equally well to the fats and to other groups. Especially in the analysis of cereals and of cattle foods, the treatment with solvents is confined to the use in successive order of ether or petroleum, alcohol, dilute acids, and alkalies, the latter at a boiling temperature. The general method of treatment with these solvents will be the subject of the following paragraphs.

48. Extraction of the Fats and Oils.—Two solvents are in general use for the extraction of fats and oils; viz., ethylic ether and a light petroleum. The former is the more common reagent.

⁶⁵ Dragendorf, *Plant Analysis*, Translated by Henry G. Greenish.

Before use it should be made as pure as possible by washing first with water, afterwards removing the water by lime or calcium chlorid, and then completing the drying by treatment with metallic sodium. The petroleum spirit used should be purified by several fractional distillations until it has nearly a constant boiling-point of from 45° to 50° . For rigid scientific determinations the petroleum is to be preferred to the ether. It is equally as good a solvent for fats and oils and is almost inert in respect to other vegetable constituents. Ether, on the other hand, dissolves chlorophyll and its partial oxidation products, resins, alkaloids and the like. The extract obtained by ether is therefore less likely to be pure fat than that secured by petroleum. For purposes of comparison, however, the ether should be employed, inasmuch as it has been used almost exclusively in analytical operations in the past.

49. Methods of Extraction.—The simplest method for accomplishing the extraction of fat from a sample consists in treating it with successive portions of the solvent in an open dish or a closed flask. This process is actually employed in some analytical operations, as, for instance, in the determination of fat in milk. Experience has shown that a portion of the substance soluble, for instance, in ether, passes very slowly into solution, so that a treatment such as that just described would have to be long continued to secure maximum results. The quantity of solvent required would thus become very large and in the case of ether would entail a great expense. For the greater number of analytical operations, some device is employed for using the same solvent repeatedly. The methods of extraction fall into two general classes; viz., extraction by digestion and extraction by percolation. This classification holds good also for other solvents besides ether and petroleum. In general, the principles and practice of extraction described for ether may serve equally well for alcohol, acetone and other common solvents.

50. Extraction by Digestion.—In the use of ether or petroleum the sample is covered with an excess of the solvent and allowed to remain for some time in contact therewith. The solu-

ble portions of the sample diffuse into the reagent. The speed of diffusion is promoted by stirring the mixtures. The operation may be conducted in an open dish or flask with proper precautions to avoid fire. Inasmuch as the residue is, as a rule, to be dried and weighed, an open dish is to be preferred. To avoid loss of reagent and to prevent filling a working room with very dangerous gases, the temperature of digestion should be kept below the boiling-point of the solvent. The greater part of the soluble matter will be extracted with three or four successive applications of the reagent, but, as intimated above, the last portions of the soluble material are extracted with difficulty by this process. In pouring off the solvent care must be exercised to avoid loss of particles of the sample suspended therein. To this end it is best to pour the solvent through a filter. For the extraction of large quantities of material for the purpose of securing the extract for future examination, or simply to remove it, the digestion process is usually employed. This excess of solvent required is easily recovered by subsequent distillation and used again. The method is rarely used for the quantitative estimation of the extract, the process of continuous percolation being more convenient and more exact. No fire, flame or other source of ignition should be allowed in any room where such extractions are conducted.

51. Extraction by Percolation.—In this method the solvent employed is poured on the top of the material to be extracted and allowed to pass through it usually by gravitation alone, sometimes with the help of a filter-pump. The principle of the process is essentially that of washing precipitates.

Two distinct forms of apparatus are in use for this process. In the first kind the solvent is poured over the material and after percolation is secured by distillation in another apparatus. In the second kind the solvent is secured after percolation in a flask where it is at once subjected to distillation. The vapors of the solvent are conducted by appropriate means to a condenser placed above the sample. After condensation the solvent is returned to the upper part of the sample. The percolation thus becomes continuous and a very small quantity of the sol-

vent may be employed to extract a comparatively large amount of material. This process is particularly applicable to the quantitative determination of the extract. After distillation and drying the latter may be weighed in the flask in which it was received or the sample may be dried and weighed in the vessel in which it is held both before and after extraction. One great advantage of the continuous extraction method lies in the fact that when it is once properly started it goes on without further attention from the analyst save an occasional examination of the flow of water through the condenser and of the rate of the distillation. For this reason the process may be continued for many hours without any notable loss of time. The vapor of the solvent in passing to the condenser may pass through a tube out of contact with the material to be extracted or it may pass directly around the tube holding the sample. In the former case the advantage is secured of conducting the extraction at a higher temperature, but there is danger of boiling the solvent in contact with the material and thus permitting the loss of a portion of the sample.

52. Apparatus Used for Extractions.—For extraction by digestion, as has already been said, an open dish may be used. When large quantities of material are under treatment, heavy flasks, holding from five to 10 liters, will be found convenient. In these cases a condenser can be attached to the flask and the extraction conducted at the boiling temperature of the solvent. During the process of extraction it is advisable to shake the flask frequently. By proceeding in this way the greater part of the solvent matter will be removed after three or four successive treatments.

In extraction by percolation various forms of apparatus are employed. The ordinary percolators of the manufacturing pharmacist may be used for the larger operations, while the more elaborate forms of continuous extractors will be found most convenient for quantitative work. In each case the analyst must choose that process and form of apparatus best suited to the purpose in view. In the next paragraphs will be described some of the more common forms of apparatus in use.

53. Knorr's Extraction Apparatus.—The apparatus which has been chiefly used by the author for the past few years is shown in the accompanying figure.⁶⁶ The principle of the construction of the apparatus lies in the complete suppression of

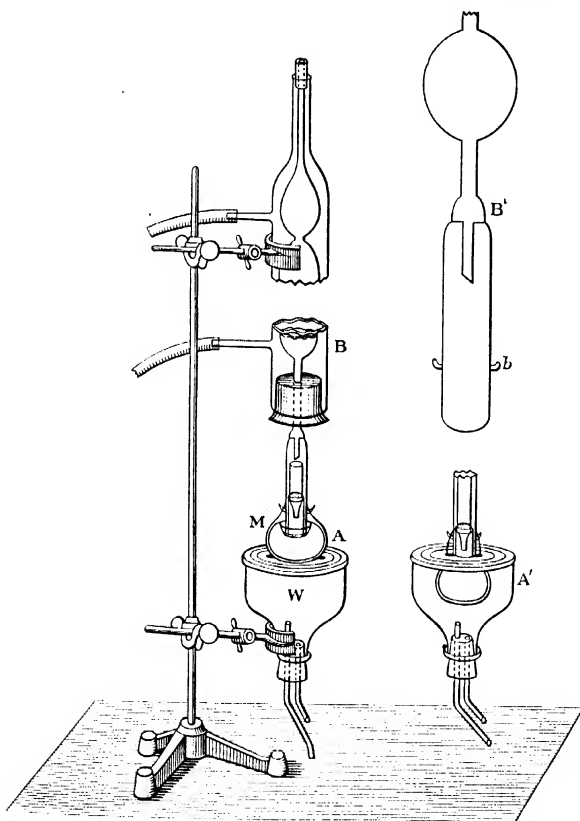


Fig. 21.—Knorr's Extraction Apparatus.

stoppers and in sealing the only joint of the device with mercury.

The construction and operation of the apparatus will be understood by a brief description of its parts.

⁶⁶ Division of Chemistry, Bulletin 28:96.

A is the flask containing the solvent, W a steam-bath made by cutting off the top of a bottle, inverting it and conducting the steam into one of the tubes shown in the stopper while the condensed water runs out of the other. The top of the bath is covered with a number of concentric copper rings, so that the opening may be made of any desirable size. B represents the condenser, which is a long glass tube on which a number of bulbs has been blown, and which is attached to the hood for holding the material to be extracted, as represented at B', making a solid glass union. Before joining the tube at B' the rubber stopper

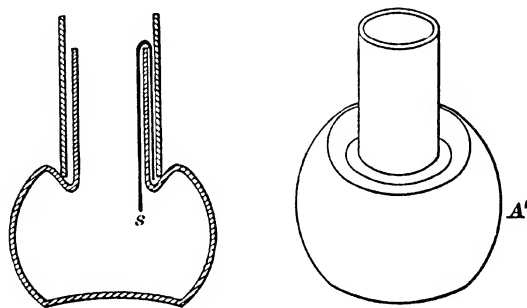


Fig. 22.—Extraction Flask.

which is to hold it into the outside condenser B is slipped on, or the rubber stopper may be cut into its center and slipped over the tube after the union is made. In case alcohol is to be used for the solvent, requiring a higher temperature, the flask holding the solvent is placed entirely within the steam-bath, as represented at A'.

A more detailed description of the different parts of the apparatus can be seen by consulting Figs. 22, 23, and 24. In A, Fig. 22, is represented a section of the flask which holds the solvent, showing how the sides of the hood containing the matters to be extracted pass over the neck of the flask, and showing at S a small siphon inserted in the space between the neck of the

flask and the walls of the hood for the purpose of removing any solvent that may accumulate in this space. A view of the flask itself is shown at A'. It is made by taking an ordinary flask, softening it about the neck and pressing the neck in so as to form a cup, as indicated at A', to hold the mercury which seals the

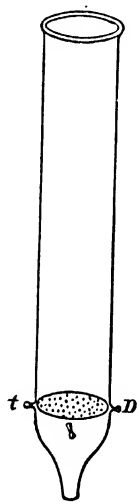


Fig. 23.—Extraction Tube.

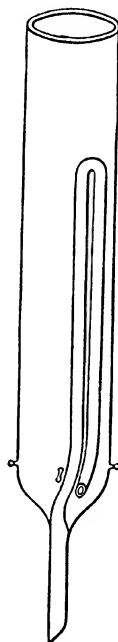


Fig. 24.—Extraction Siphon Tube.

union of the flask with the condenser. The flask is held in position by passing a rubber band below it, which is attached to two glass nipples, *b*, blown onto the containing vessel, as shown in Fig. 21. The material to be extracted may be contained in an ordinary tube drawn out, as indicated in Fig. 23, having a perforated platinum disk sealed in at D. The containing tube rests upon the edges of the flask containing the solvent by means of

nipples shown at *t*. If a siphon tube is to be used, one of the most convenient forms is shown in Fig. 24, in which the siphon lies entirely within the extracting tube, thus being protected from breakage. By means of this apparatus the extractions can be carried on with a very small quantity of solvent, there being scarcely any leakage, even with the most volatile solvents, such as ether and petroleum. The apparatus is always ready for use,



Fig. 25. Battery of Knorr's Extractors in Bureau of Chemistry.

(Photo by Doyle.)

no corks are to be extracted, and no ground glass joints to be fitted. Where many of these apparatus are to be used at the same time or continuously the extraction flasks are conveniently made to rest on a heating surface of thick iron plate electrically warmed to the proper temperature and the heat regulated by a thermostat. An efficient apparatus of this kind is used in the Bureau of Chemistry and one side of the extraction room show-

ing the electrically heated plates on which the ether flasks rest is shown in Fig. 25.

54. Soxhlet's Extraction Apparatus.—A form of continuous extraction apparatus has been proposed by Soxhlet which permits the passage of the vapors of the solvent into the condenser by a separate tube and the return of the condensed solvent after hav-



Fig. 26.—Soxhlet Extraction Apparatus.

ing stood in contact with the sample, to the evaporating flask by a siphon. The advantage of this process lies in freeing the sample entirely from the rise of temperature due to contact with the vapors of the solvent, and in the second place in the complete saturation of the sample with the solvent before siphoning. The sample is conveniently held in a cylinder of extracted filter-paper open above and closed below. This is placed in the large tube between the evaporating flask and the condenser. The sample should not fill the paper holder, and if disposed to float in the solvent, should be held down with a plug of asbestos fiber or of

glass-wool. The extract may be transferred, by dissolving in the solvent, from the flask to a drying dish, or it may be dried and weighed in the flask where first received.

There are many forms of apparatus of this kind, one of which is shown in Fig. 26, but a more extended description of them is not necessary. The disadvantages of this process as compared with Knorr's, are quite apparent. The connections with the evaporating flask and condenser are made with cork stoppers, which must be previously thoroughly extracted with ether and alcohol. These corks soon become dry and hard and difficult to use. The joints are likely to leak, and grave dangers of explosions arise from the vapors of the solvents escaping into the working room. Moreover, it is an advantage to have the sample warmed by the vapors of the solvent during the progress of the extraction, provided the liquid in direct contact with the sample does not boil with sufficient vigor to cause loss.

The use of extraction apparatus with ground glass joints is also unsatisfactory. By reason of unequal expansion and contraction these joints often are not tight. They are also liable to break and thus bring danger and loss of time.

55. Compact Extraction Apparatus.—In order to bring the extraction apparatus into a more compact form, the following described device has been successfully used.⁶⁷ The condenser employed is made of metal and is found entirely within the tube holding the solvent.

This form of condenser is shown in Fig. 27, in which the tube E serves to introduce the cold water to the bottom of the condensing device. The tube D serves to carry away the waste water. The tube F serves for the introduction of the solvent by means of a small funnel. When the solvent is introduced and has boiled for a short time, the tube F should be closed. In each of the double conical sections of the condenser a circular disk B is found, which causes the water flowing from A upward to pass against the metallic surfaces of the condenser.

A section of the double conical condenser is shown in the

⁶⁷ Journal of Analytical and Applied Chemistry, 1893, 7: 65.

upper right hand corner. It is provided with two small hooks *hh*, soldered on the lower surface, by means of which the crucible *G* can be hung with a platinum wire. The condenser is best made smooth and doubly conical in form.

The crucible *G*, which holds the material to be extracted, can

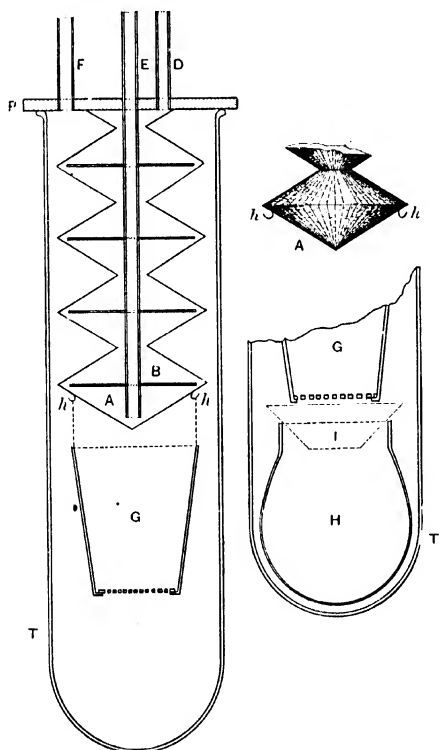


Fig. 27.- Compact Condensing Apparatus.

be made of platinum, but for economy porcelain may be used. The bottom of the porcelain crucible is left open except for a small shelf, as indicated, which supports a perforated disk of platinum on which an asbestos film is placed.

The whole apparatus is of such size as to be easily contained in the large test-tube *T*.

The mouth of the test-tube is ground so as to fit as smoothly as possible on the ground brass plate of the metallic condenser P.

In case it is desired to weigh the extract it may be done directly by weighing it in the test-tube T after drying in the usual way at the end of the extraction; or a glass flask H, made to fit freely into the test-tube, may be used, in which case a little mercury is poured into the bottom of the tube to seal the space between H and T. To prevent spirting of the substance in H, or projecting any of the extracted material without or against the bottom of the crucible G, the funnel represented by the dotted lines in the right-hand section may be used.

Heat may be applied to the test-tube either by hot water, or steam, or, by a bunsen, which permits of the flame being turned down to minimum proportions without danger of burning back. When the test-tube alone is used it is advisable to first put into it some fragments of pumice stone, particles of platinum foil, or a spoonful of shot, to prevent bumping of the liquid when the lamp is used as the source of heat.

Any air which the apparatus contains is pushed out through F when the boiling begins, the tube F not being closed until the vapor of the liquid has reached its maximum height. With cold water in the condenser the vapor of ether very rarely reaches above the lower compartment and the vapor of alcohol rarely above the second.

When the plate P is accurately turned so as to fit the ground surface of the mouth of T, it is found that 10 cubic centimeters of anhydrous ether or alcohol are sufficient to make a complete extraction, and there is not much loss of solvent in six hours. The thickness of the asbestos film in G, or its fineness, is so adjusted as to prevent too rapid filtration so that the solvent may just cover the material to be extracted, or, after the material is placed in a crucible, a plug of extracted glass-wool may be placed above it for the purpose of distributing the solvent evenly over the surface of the material to be extracted and of preventing the escape of fine particles.

In very warm weather the apparatus may be arranged as shown in Fig. 28. The bath for holding the extraction tubes

is made in two parts, K and K'. The bath K has a false bottom shown in the dotted line O, perforated to receive the ends

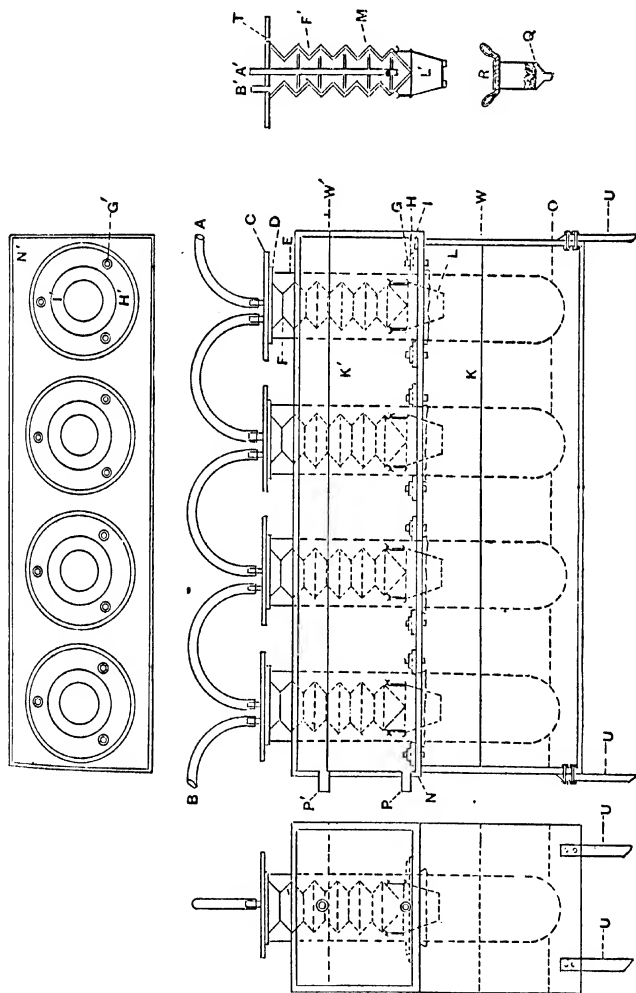


Fig. 28.—Improved Compact Extraction Apparatus.

of the extraction tubes and which holds them in place and pre-

vents them from touching the true bottom, where they might be unequally heated by the lamp. The upper bath K' has a perforated bottom, partly closed with rubber cloth diaphragms G' N' H'. The extraction tubes passing through this bath, water-tight, permit broken ice or ice-water to be held about their tops, and thus secure a complete condensation of the vapors of the solvent which in warm weather might escape the metal condenser. In practice care must be taken to avoid enveloping too much of the upper part of the extraction tube with the ice-water, otherwise the vapors of the solvent will be chiefly condensed on the sides of the extraction tube and will not be returned through the sample. It is not often that the upper bath is needed, and then only with ether, never with alcohol.

The apparatus without the upper part has proved especially useful with alcohol, using, as suggested, glycerol in the bath. The details of its further construction and arrangement are shown in the figure. The extraction tubes are most conveniently arranged in a battery of four, one current of cold water passing in at A and out at B, serving for all. The bath is supported on legs long enough to allow the lamp plenty of room. The details of the condenser M are shown in B', A', T, I', and I'. Instead of a gooch I' for holding the sample a glass tube R, with a perforated platinum disk Q, may be used. The water line in the bath is shown by W. This apparatus may be made very cheaply and without greatly impairing its efficiency by using a plain concentric condenser and leaving off the upper bath K'.

56. Solvents Employed.—It has already been intimated that the chief solvents employed in the extraction of agricultural samples are ether, petroleum ether and alcohol. The ether used should be free of alcohol and water, the petroleum should be subjected to fractional distillation to free it of the parts of very high and very low boiling-points, and the alcohol may contain about 20 per cent. of water for many purposes.

There are many instances where other solvents should be used. The use of aqueous alcohol is sometimes preceded by that of alcohol of greater strength or practically free of water. For

the extraction of soluble carbohydrates (sugars) cold or tepid water is employed, the temperature of which is not allowed to rise high enough to act upon starch granules. For the solution of the starch itself an acid solvent is used at a boiling temperature, whereby the starch molecules undergo hydrolysis and form dextrin or soluble sugars (maltose, dextrose). By this process also the carbohydrates, whose molecules contain five, or some multiple thereof, atoms of carbon, form soluble sugars of which xylose and arabinose are types. The solvent action of acids followed by treatment with dilute alkalies at a boiling temperature, completes practically the solution of all the carbohydrate bodies, save cellulose and nearly related compounds. The starch carbohydrates are further dissolved by the action of certain ferments such as diastase.

Dilute solutions of mineral salts exert a specific solvent action on certain nitrogenous compounds and serve to help separate the proteid bodies into definite groups.

Under the proper headings the uses of these principal solvents will be described, but a complete discussion of their action, especially on samples of a vegetable origin, should be looked for in works on plant analysis.

The application of acids and alkalies for the extraction of carbohydrates, insoluble in water and alcohol, will be described, in the paragraphs devoted to the analysis of fodders and cereals. The extraction of these matters, made soluble by ferments, will be discussed in the pages devoted to starch and artificial digestion. It is thus seen that the general preliminary treatment of a sample preparatory to specific methods of examination is confined to drying, extraction with ether and alcohol, and incineration.

In general it may be said that in the use of any given solvent the rapidity and completeness of the process at any stated temperature are greatly promoted by the fineness of particles and degree of porosity of the sample. Both with ether and petroleum ether the process is greatly facilitated by the complete absence of water.

57. Recovery of the Solvent.—In using such solvents as ether,

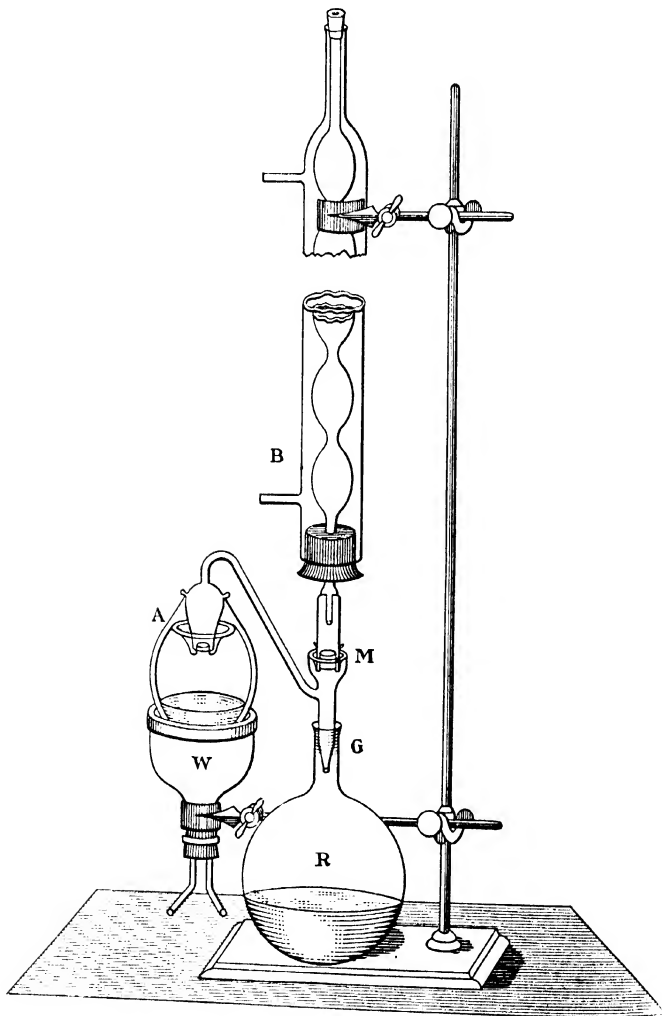


Fig. 29.—Knorr's Apparatus for Recovering Solvents.

chloroform, and others of high value, it is desirable often to

recover them. Various forms of apparatus are employed for this purpose, arranged in such a way as both to secure the solvent and to leave the residue in an accessible condition, or in a form suited to weighing in quantitative work. When the ex-

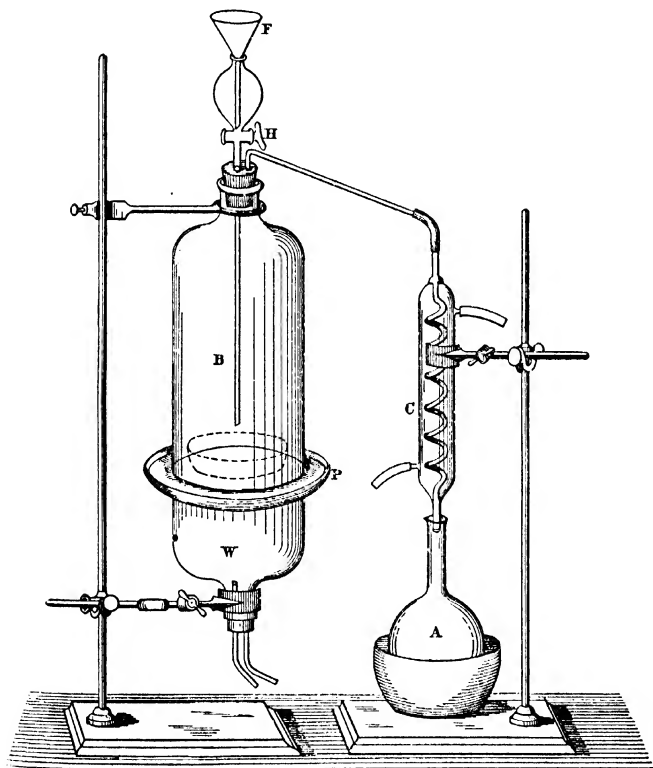


Fig. 30.—Apparatus for Recovering Solvents from Open Dishes.

tractions are made according to the improved method of Knorr, the flask containing the extract may be at once connected with the apparatus shown in Fig. 29.⁶⁸ A represents the flask containing the solvent to be recovered, W the steam-bath, B the

⁶⁸ Division of Chemistry, Bulletin 28:99.

condenser sealed by mercury, M the union of the tube delivering the vapor to the condenser, also sealed with mercury and R the flask receiving the products of condensation. The open end of the connecting tube, which is sealed on A by mercury, should be the same size as the tube connecting with the condenser in the extraction apparatus. It will be found economical to save ether, alcohol, and chloroform even when only a few cubic centimeters remain after the extraction is complete.

It often happens that the materials which are dissolved by the ordinary solvents in use are to be collected in open dishes in order that their properties may be studied. At the same time large quantities of solvents must be used, and it is desirable to have some method of recovering them. The device shown in Fig. 30 has been found to work excellently well for this purpose.⁶⁹ It consists of a steam-bath, W, and a bottle, B, with the bottom cut off, resting on an iron dish, P, containing a small quantity of mercury, enough to seal the bottom of the bottle. The dish containing the solvent is placed on the mercury, and the bottle placed down over it, forming a tight joint. On the application of steam the solvent escapes into the condenser, C, and is collected as a liquid in the flask A. With very volatile solvents the flask A may be surrounded with ice, or ice-cold water passed through the condenser. When an additional quantity of the solvent is to be added to the dish for the purpose of evaporation it is poured into the funnel F, and the stop-cock H opened, which allows the material to run into the dish in B without removing the bottle. In this way many liters of the solvent may be evaporated in any one dish, and the total extractions obtained together. At the last the bottle B is removed, and the extract which is found in the dish is ready for further operations.

⁶⁹ Division of Chemistry, Bulletin 28: 103.

PART SECOND

SUGARS AND STARCHES

58. Introduction.—Carbohydrates, of which sugars and starches are the chief representatives, form the great mass of the results of vegetable metabolism. The first functions of the chlorophyll cells of the young plant are the condensation of carbon dioxid and water. The simplest form of the condensation is formaldehyd, CH_2O . There is no convincing evidence, however, that this is the product resulting from the functional activity of the chlorophyll cells. The first evidence of the condensation is found in more complex molecules; *viz.*, those having six atoms of carbon. It is not the purpose of this work to discuss the physiology of this process, but the interested student can easily find access to the literature of the subject.⁷⁰ When a sample of a vegetable nature reaches the analyst he finds by far the largest part of its substance composed of these products of condensation of the carbon dioxid and water. The sugars, starches, pentosans, lignoses, and celluloses all have this common origin. Of many air-dried plants these bodies form more than eighty per cent.

In green plants the sugars exist chiefly in the sap. In plants cut green and quickly dried by artificial means the sugars are found in a solid state. They also exist in the solid state naturally in certain sacchariferous seeds. Many sugar bearing plants when allowed to dry spontaneously lose all or the greater part of their sugar by fermentation or by the action of enzymes. This is true of sugar-cane, sorghum, maize stalks, and the like. The starches are found deposited chiefly in tubers, roots or seeds. In the potato the starch is in the tuber, in cassava the tuber holding the starch is also a root, in maize, rice and other cereals the starch is in the seeds. The wood-fibers, *viz.*, pentosans, lignose, cellu-

⁷⁰ Vines, *Physiology of Plants*: 143. Green, *Introduction to Vegetable Physiology*, 1907: 151.

lose, etc., form the framework and support of the plant structure. Of all these carbohydrate bodies the most important as foods are the sugars and starches, but a certain degree of digestibility cannot be denied to other carbohydrate bodies with the possible exception of pure cellulose. In the following paragraphs the general principles of determining the sugars and starches will be given and afterwards the special processes of extracting these bodies from vegetable substances preparatory to quantitative determination.

59. Nomenclature.—At first only glucoses, sucrose and polysaccharids were known but by the work of Fischer and others many isomers were found, also compounds both richer and poorer in carbon atoms than the $C_6H_{12}O_6$ or glucose.⁷¹ Attention is called to use of the term glucose here in its strictly scientific meaning and not its commercial usage which is entirely different. The general group of carbohydrates may be divided into the monosaccharids, disaccharids, trisaccharids, tetrasaccharids, and polysaccharids. These again are divided in the case of monosaccharids into bioses, trioses, tetroses, pentoses, hexoses, etc., depending upon the number of carbon atoms found in the molecule, and these latter are again divided into the individual sugars. Another division of the individual sugars is into ketoses and aldoses, depending upon the occurrence of the ketone or aldehyde group in their structure. The physical and chemical properties of the principal sugars will be dealt with later on in the work.

60. Properties of Carbohydrates.—In general the sugars have a sweet taste, are crystalline in structure and soluble to a greater or less extent in water and alcohol. They have the power of rotating the plane of polarized light and forming salts with some of the metals and also with some of the acids and various other combinations which serve in part to differentiate them.

The starches and allied bodies are amorphous in structure, rarely showing any crystalline form. They are devoid of any sweet taste and in general are optically active.

⁷¹ Fischer, *Untersuchungen über Kohlenhydrate*, 1884–1908. von Lippmann, *Die Chemie der Zuckerarten*, 3d Edition, 1904: 1–41.

61. Particular Signification of Names.—The new nomenclature as proposed by Fischer while not generally used in popular and semi-popular literature nor by some writers of scientific works, has been adopted in most books on sugar and by most sugar chemists.

The word "sugar" applies to the general class of sugars and when spoken of in the singular means the commercial product as derived from the sugar beet, the sugar cane, or the sugar palm. The term "sucrose" applies to the principal sugar in the three mentioned above. "d-Glucose" is dextrose, "fructose" is levulose, while "invert sugar" is the name applied to the product coming from the complete inversion of sucrose or the mixture of equal parts of glucose and fructose. "Reducing sugar" is the name applied to any sugar having the property of reducing alkaline copper solutions. At first the use of the word "glucose" and "fructose" for the old names "dextrose" and "levulose" may be confusing to some as the latter names are still used by many, but this nomenclature serves to bring the student into a better conception of modern theories. This confusion will be noted more particularly with the use of the term "glucose" as at present there are many substances indicated by this name. For instance "glucose" is the name applied to the viscous liquid product coming from the partial conversion of starch by acid, but this product may be called a commercial glucose in distinction from glucose and is so called by many. Again "glucose" is applied to the reducing sugars present in sugar-cane. This is quite common with the manufacturers or those interested in the manufacture of cane-sugar, but this product is rightly termed "reducing sugars," as it will be afterwards shown to be a mixture of the reducing sugars, glucose and fructose.

From a strictly scientific point of view it is advisable to adhere to the above proposed terminology. In point of fact, however, no confusion will arise from the analytical point of view by using the terms, "dextrose," "levulose," etc., and these terms are generally used in the following pages.

62. Classification of Methods.—In the quantitative determination of carbohydrates the various processes employed may be grouped into the three general classes. First, densimetric methods; second, polarimetric methods; and third, chemical methods. This order will be followed. Under each a full description will be given of the ordinary processes, employed together with the principles on which they are based in so far as not to depart from the main purpose of this work. Following this will be considered some of the most common sugars and their properties, together with the tests for their identification and means for separating and identifying them in the presence of other sugars in so far as the subject has developed. The methods for the identification of these bodies are by no means complete. The final chapter contains the methods for determining carbohydrates in various agricultural products.

THE DETERMINATION OF THE PERCENTAGE OF SUGAR BY THE DENSITY OF ITS SOLUTION.

63. Principles of the Method.—This method of analysis may be used for any sugar but is applied almost exclusively to the examination of sucrose. The method is accurate only when applied to solutions of pure sugars which contain no other bodies since it is evident that other bodies in solution will affect the density in much the same way as pure sugars.

Broadly stated the principle of the method consists in determining the specific gravity of the liquid solution, and thereafter taking the percentage of the body in solution from the corresponding specific gravity in a table. These tables are called Balling, Brix and Baumé, depending on the name of the inventor of the system used. Sugar chemists express the density of a solution as the degree Brix, Balling or Baumé, as the case may be. These tables have been carefully prepared by gravimetric determinations of the bodies in solution of known densities, varying by small amounts and the calculation of the percentages for the intervening increments or decrements of density. This tabulation is accomplished at definite temperatures and the process

of analysis secured thereby is rapid and accurate, with pure or nearly pure solutions.

64. Determination of Density.—While not strictly correct from a physical point of view, the terms density and specific gravity are here used synonymously and refer to a direct comparison of the weights of equal volumes of pure water and of the solution in question, at the temperature named or to a volume of water at its maximum density. Much confusion exists as to the standard temperatures for taking specific gravity. In sugar work up to the present time $17^{\circ}.5$ has been chosen almost exclusively although tables based on $15^{\circ}.0$ and also 20° will be found. However the latter temperature has now been adopted almost universally and the gravity is referred to water at 4° . The expression for this is specific gravity at $20^{\circ}/4^{\circ}$. When other temperatures are employed, they may be referred to water of the same temperature, and the expression of the specific gravity is then $17^{\circ}.5/17^{\circ}.5$ or $15^{\circ}/15^{\circ}$. It is these varying standards of interpretation of the temperature that cause confusion in the tables.

The standard or most accurate method of determining the density of a solution is to get the weight of a definite volume thereof. This is conveniently accomplished by the use of a pyknometer. A pyknometer is any vessel capable of holding a definite volume of a liquid in a form suited to weighing. It may be a simple flask with a narrow neck distinctly marked, or a flask with a ground perforated stopper, which, when inserted, secures always the same volume of liquid contents. A very common form of pyknometer is one in which the central stopper carries a thermometer and the constancy of volume is secured by a side tubulure of very small even capillary dimensions, which is closed by a ground glass cap.

The apparatus may not even be of flask form, but assume a quite different shape as in Sprengel's tube. This is the form used for very accurate work. Pyknometers are often made to hold an even number of cubic centimeters, but the only advantage of this is in the ease of calculation which it secures. As a rule, it will be found necessary to calibrate even these, and then

the apparent advantage will be easily lost. A flask which is graduated to hold 50 cubic centimeters, may, in a few years, change its volume at least slightly, due to molecular changes in the glass. Two of the many forms of pyknometers are shown in the accompanying figures.

In use the pyknometer should be filled with recently boiled and cooled distilled water of the desired temperature and weighed.

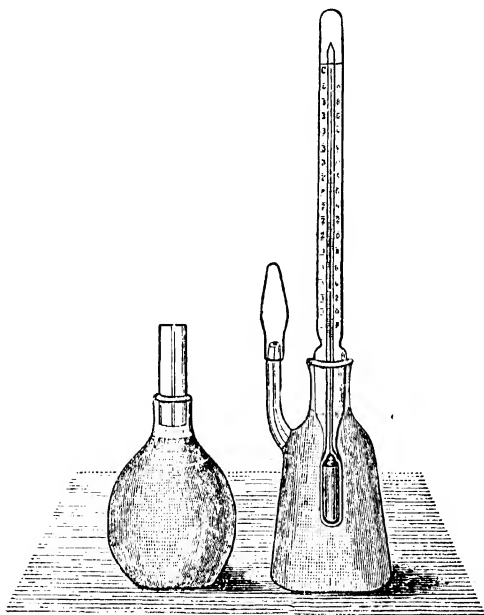


Fig. 31. Common Forms of Pycnometers.

From the total weight the tare of the flask and stopper, weighed clean and dry, is to be deducted. The remainder is the weight of the volume of water of the temperature noted, which the pycnometer holds. The weight of the solution under examination is taken in the same way and at the same temperature, and thus a direct comparison between the two liquids is secured.

Example.—Let the weight of the pyknometer be. 15.2985 grams.
and its weight with pure water at 15.5° be.....26.9327 “

Then the weight of water is.....11.6342 “

The weight filled with the sugar solution is.....28.3263 “

Then the weight of the sugar solution is.....13.0278 “

The specific gravity of the sugar solution is therefore, $13.0278 \div 11.6342 = 1.1198$ at 15.5° compared with water at 15.5°.

For strictly accurate results the weight must be corrected for the volume of air displaced, or in other words, be reduced to weights in vacuo. This however is unnecessary for the ordinary operations of agricultural analysis.

If the volume of the pyknometer be desired, it can be calculated from the weight of pure water which it holds, one cubic centimeter of pure water weighing one gram at 4°.

The weights of one cubic centimeter of water at each degree of temperature from 1° to 40°, are given in the following table:

TABLE SHOWING WEIGHTS OF ONE CUBIC CENTIMETER OF PURE WATER
AT TEMPERATURES VARYING FROM 1° TO 40°.

Temperature.	Weight, Gram.	Temperature.	Weight, Gram.
0°	0.99987	21°	0.99802
1°	0.99993	22°	0.99780
2°	0.99997	23°	0.99756
3°	0.99999	24°	0.99732
4°	1.00000	25°	0.99707
5°	0.99999	26°	0.99681
6°	0.99997	27°	0.99654
7°	0.99993	28°	0.99626
8°	0.99988	29°	0.99597
9°	0.99981	30°	0.99567
10°	0.99973	31°	0.99537
11°	0.99963	32°	0.99505
12°	0.99952	33°	0.99473
13°	0.99940	34°	0.99440
14°	0.99927	35°	0.99406
15°	0.99913	36°	0.99371
16°	0.99897	37°	0.99336
17°	0.99880	38°	0.99299
18°	0.99862	39°	0.99262
19°	0.99843	40°	0.99224
20°	0.99823		

From the table and the weight of water found, the volume of the pycnometer is easily calculated.

Example.—Let the weight of water found be 11.7289 grams, and the temperature 20°. Then the volume of the flask is equal to $11.7289 \div 0.99823$, viz., 11.74 cubic centimeters.

65. Use of Pycnometer at High Temperatures.—It is often found desirable to determine the density of a liquid at temperatures above that of the laboratory, *e. g.*, at the boiling-point of water. This is easily accomplished by following the directions given below:

Weight of Flask.—Use a small pycnometer of from twenty-five to thirty cubic centimeters capacity. The stopper should be beveled to a fine edge on top and the lower end should be slightly concave to avoid any trapping of air. The flask is to be thoroughly washed with hot water, alcohol and ether, and then dried for some time at 100°. After cooling in a desiccator the weight of the flask and stopper is accurately determined.⁷²

Weight of Water.—The flask in an appropriate holder. Fig. 32 conveniently made of galvanized iron, is filled with freshly boiled and hot distilled water and placed in a bath of pure, very hot distilled water, in such a way that it is entirely surrounded by the liquid with the exception of the top.

The water of the bath is kept in brisk ebullition for thirty minutes, any evaporation from the flask being replaced by the addition of boiling distilled water. The stopper should be kept for a few minutes before use in hot distilled water and is then inserted, the flask removed, wiped dry, and, after it is nearly cooled to room temperature, placed in the balance and weighed when balance temperature is reached. A convenient size of holder will enable the analyst to use eight or ten flasks at once. The temperature at which water boils in each locality may also be determined; but unless at very high altitudes, or on days of unusual barometric disturbance the variations will not be great, and will not appreciably affect the results.

⁷² Bulletin, 28, Division of Chemistry, U. S. Department of Agriculture: 197.

66. Determination of Specific Gravity at $\frac{20^{\circ}}{4^{\circ}}$ C. — The pyknometer is filled with recently boiled distilled water and placed

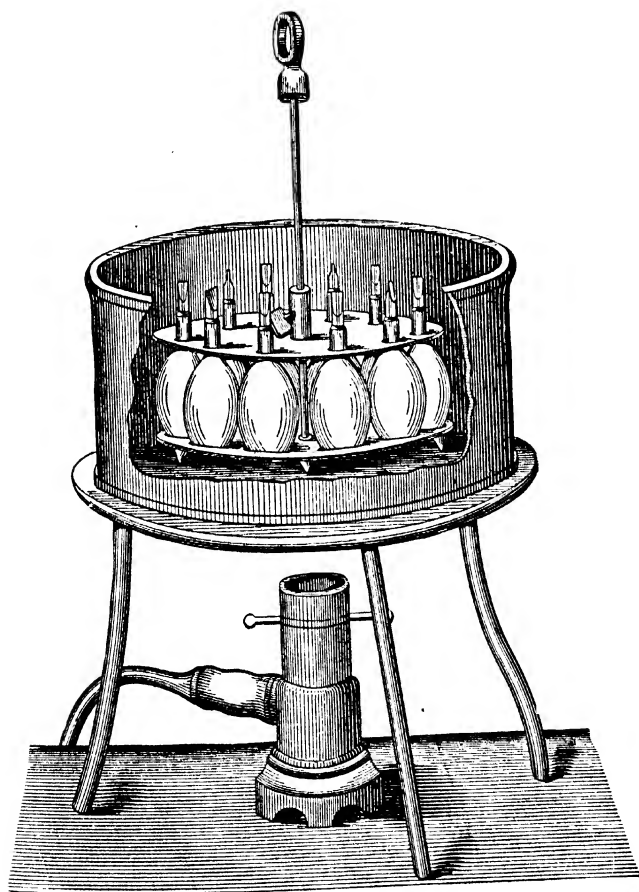


Fig. 32.—Bath for Pyknometers.

in a water-bath of constant temperature at approximately 20° C. When the water in the pyknometer has reached the same tem-

perature as the water of the bath, the liquid is adjusted to the marks of the pyknometer which is then closed and removed from the bath. The temperature of the water having been accurately determined. After wiping dry and allowing the water to come to the temperature of the room the pyknometer is weighed. The weight of the empty dry pyknometer having been previously ascertained, it is subtracted from this weight. The result is the weight of water at the temperature taken. The same pyknometer is again filled with the sugar solution and the operation repeated in detail as before and at same temperature as that of the water. By subtraction the weight of the solution is obtained. The calculations are made according to the formula given in Landolt.⁷³

$$d_t = \frac{F Q_0}{W_0} + \frac{F Q_0}{W_0} 3 \beta (t_0 - t) - \frac{\lambda (F - W_0)}{W_0}$$

In which

t_0 = temperature at which readings were made in °C.

W_0 = the apparent mass of the water in the air at the temperature t_0 .

F = the apparent mass of the sugar solution in the air at the temperature t .

Q_0 = is found from table following and is the specific gravity of water at the temperature t_0 .

3β = 0.000024, the coefficient of cubical expansion of glass.

λ = 0.0012, the air density.

d_t = specific gravity of the solution of the sugar at the temperature t referred to water at 4° C.

If the differences between t and t_0 are small, a mean value 0.000024 may be used for 3β .

⁷³ Optical rotation of Organic Substances, Long's translation, 1902: 454.

SPECIFIC GRAVITY OF WATER AT VARIOUS TEMPERATURES¹⁴.

<i>t</i> ₀	<i>Q</i> ₀	<i>t</i> ₀	<i>Q</i> ₀	<i>t</i> ₀	<i>Q</i> ₂
10.0	0.999731	19.0	0.998437	21.6	0.997890
11.0	637	.1	417	.7	868
12.0	530	.2	397	.8	846
13.0	410	.3	377	.9	823
14.0	277	.4	357	22.0	800
15.0	132	.5	337	.1	778
16.0	0.998976	.6	317	.2	755
17.0	808	.7	296	.3	732
.1	790	.8	276	.4	709
.2	772	.9	255	.5	685
.3	755	20.0	235	.6	662
.4	737	.1	214	.7	639
.5	719	.2	193	.8	615
.6	701	.3	172	.9	592
.7	683	.4	151	23.0	568
.8	664	.5	130	24.0	546
.9	646	.6	109	25.0	523
18.0	628	.7	087	26.0	0.996811
.1	609	.8	066	27.0	500
.2	590	.9	044	28.0	476
.3	571	21.0	023	29.0	0.995971
.4	552	.1	001	30.0	674
.5	533	.2	0.997979	31.0	368
.6	514	.3	957		
.7	495	.4	935		
.8	476	.5	913		
.9	456				

87. Determination of Density by the Westphal Balance.—

While the pycnometer is useful in control work and in fixing standards of comparison, it is not used extensively in practical work. Quicker methods of determination are desired in such work, and these are found in the use of other forms of apparatus. A convenient method of operation consists in having a glass bob so adjusted as to be capable of displacing a given number of grams (five for instance) of distilled water at a given temperature when wholly immersed in the liquid and suspended by a fine platinum wire, immersed to a given depth. The instrument devised by Mohr and modified by Westphal, is based upon that

¹⁴ Landolt and Böernstein's Tables, 1894 : 37.

principle, and is used somewhat in practical work although its use cannot be highly recommended for accuracy. The construction of this apparatus is shown in Fig. 33.

The arm is provided with a knife edge near the center. On one end is a hook to receive the bob and this end of the knife edge is

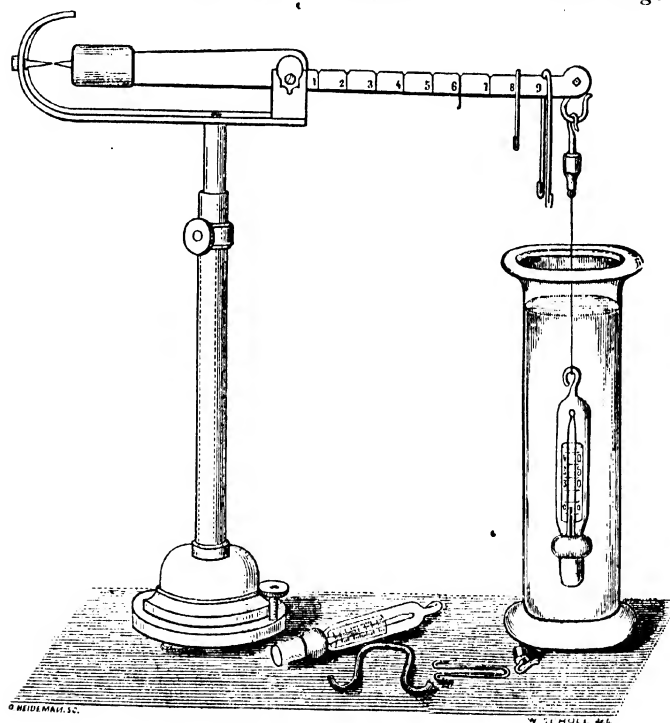


Fig. 33. Westphal's Balance.

divided into 10 sections. The other end has a counter poise weight with pointer. The bob is put in place and suspended in water and the large weight is placed on the same hook. The leveling screw at the base is then turned until the point on arm and on frame coincide where the former comes to rest after being vibrated. When this is set, the vessel is filled with the sugar solution and the varying weights placed along the beam in

the notches until equilibrium is established. From the size of weights and their place on the beam the specific gravity is read. Care must be used to have the solution at the temperature the balance is graduated to obtain a correct reading.

68. The Areometric Method.—A rapid method of determining the density of a solution and the one in most common use, is based on the distance to which a heavy bulb with a slender graduated stem will sink therein. An instrument of this kind is called a hydrometer, a specific gravity, Brix, or Baumé spindle, a saccharometer, alcoholometer, etc., depending on the use for which it is constructed.

In general hydrometers are made of glass and consist of three parts, the scale, a rather thick and short bulb, and a small bulb filled with mercury, or in some cases shot. They are graduated by placing them in solutions of known density and marking on stem the point to which they sink. Two or three of these points are determined along the stem and then the intermediate points gotten by interpolation. In determining the point to which they sink, the reading is made not at the top of the meniscus but at the level of the solution. When reading very dark solutions this point has very often to be estimated. In reading and using these instruments care must be taken to see that the vessel holding the solution is large enough to prevent the hydrometer from sticking to the side and to permit it to move freely. The portion of the hydrometer exposed to the air must be dry. The reading should only be made after the hydrometer has had time to come to rest and assume the temperature of the solution. This temperature should be the one at which the hydrometer is graduated but for some of the scales a table of temperature corrections has been prepared.

In sugar work the Brix scale is used generally in this country, although some use is made of the Baumé scale.

69. Balling Scale.—This scale was the original one prepared for sugar solutions by an Austrian chemist, Balling. He made solutions of sucrose by dissolving 1, 5, 10, 15, 20 grams etc., in 99, 95, 90, 85, and 80 grams of water respectively and obtained

the specific gravity of each. In this way a table was prepared showing the specific gravity for solutions of varying amounts of sugar. The Balling reading expressed usually as degrees, is the weight of sucrose in 100 grams of the solution: Inaccuracies were found in this table and a recalculation of the data was made by Brix, a German chemist. In Germany the term Balling as now applied is synonymous with Brix.

70. Brix Scale.—This scale is the one generally used in sugar work. The degrees Brix represent the per cent. by weight of sucrose in a solution. In sugar house control this has come to mean the per cent. of total solids in a solution. The specific gravity equivalents of the degrees Brix in published tables very considerably depending on the temperature at which they were taken. Mateczek and Scheibler have calculated a table for 17°.5/17°.5 and Scheibler for 15°/15° and Plato for 20°/4°.

71. Baume' Scale. There are possibly more tables in existence for this scale than for any other. Chandler⁷⁵ found 36 different scales in use, many of them incorrect. As originally constructed the point to which the instrument sank when immersed in a 10 per cent. solution by weight of common salt in water was taken as 10°. The interval between this point and that at which the hydrometer stood when immersed in pure water was divided into 10 equal parts, and a scale of similar equal parts extended as far as was necessary. Lunge⁷⁶ gives the following formula for converting Baumé degrees obtained by reference to a 10 per cent. salt solution into specific gravity, n representing the observed degree.

	Liquids heavier than water		Liquids lighter than water
At 12.5°, sp. gr. =	$\frac{145.88}{145.88 - n}$	sp. gr. =	$\frac{145.88}{133.88 + n}$
At 15.0°, sp. gr. =	$\frac{146.3}{146.3 - n}$	sp. gr. =	$\frac{146.3}{136.3 + n}$
At 17.5°, sp. gr. =	$\frac{146.78}{146.78 - n}$	sp. gr. =	$\frac{146.78}{136.78 + n}$

⁷⁵ Proceedings National Academy of Science, 1881, 3.

⁷⁶ Technical Methods of Chemical Analysis translated by Kearn. 1. Part 1: 158.

The "rational" hydrometer proposed by Kolb in France, but widely used in Germany sinks in distilled water at the standard temperature to zero, and in pure sulfuric acid of sp. gr. 1.8427 at 15° sinks to 66°. With this the specific gravity = $\frac{144.3}{144.3 - n}$.

The table recommended for solutions heavier than water by the Bureau of Standards⁷⁷ is calculated from the formula

$$\text{Degrees Baumé} = 145 - \frac{145}{D_{60^{\circ}}^{\text{60}^{\circ}} F}$$

For solutions lighter than water, the following formula is used:

$$\text{Degrees Baumé} = \frac{140}{D_{60^{\circ}}^{\text{60}^{\circ}} F} - 130.$$

The Association of Official Agricultural Chemists⁷⁸ have also adopted this.

As can be seen from this discussion the Baumé scale has nothing to do with sugar solutions. It finds use in sugar house work as a rough measure of the density of juices and especially thickened sirups

72. Brix, Baume' and Specific Gravity Tables.—In the following tables taken from Rumpler with a few modifications are found the degree Brix, the degree Baumé and the specific gravity numbers from pure water to solid sugar.⁷⁹ The limit of the solubility of sugar is a solution of about 66 per cent. From this point on the data are obtained by calculation. The specific gravity equivalents of degrees Brix and Baumé are given for $\frac{17^{\circ}.5}{17^{\circ}.5}$ the table in usual use and $\frac{20^{\circ}}{4}$ the table recommended by most sugar chemists.

⁷⁷ Circular 19. Bureau of Standards : 16.

⁷⁸ Bureau of Chemistry. Bulletin 107, revised : 221.

⁷⁹ Aus führliches Handbuch der Zucker-Fabrikation, 1906.

° Brix	° Baumé	Specific gravity		° Brix	° Baumé	Specific gravity	
		20° 4°	17.5° 17.5°			20° 4°	17.5° 17.5°
0.0	0.00	0.998234	1.00000	21.5	12.13	1.087652	1.09004
0.5	0.28	1.000174	1.00193	22.0	12.40	1.089900	1.09231
1.0	0.57	1.002120	1.00388	22.5	12.68	1.092155	1.09458
1.5	0.85	1.004064	1.00583	23.0	12.96	1.094420	1.09686
2.0	1.14	1.006015	1.00779	23.5	13.24	1.096691	1.09915
2.5	1.42	1.007972	1.00976	24.0	13.52	1.098971	1.10145
3.0	1.70	1.009934	1.01173	24.5	13.80	1.101259	1.10375
3.5	1.99	1.011904	1.01371	25.0	14.08	1.103557	1.10607
4.0	2.27	1.013881	1.01570	25.5	14.35	1.105862	1.10839
4.5	2.55	1.015864	1.01770	26.0	14.63	1.108175	1.11072
5.0	2.84	1.017854	1.01970	26.5	14.91	1.110497	1.11306
5.5	3.12	1.019851	1.02171	27.0	15.19	1.112828	1.11541
6.0	3.40	1.021855	1.02373	27.5	15.46	1.115166	1.11776
6.5	3.69	1.023867	1.02575	28.0	15.74	1.117512	1.12013
7.0	3.97	1.025885	1.02779	28.5	16.02	1.119867	1.12250
7.5	4.25	1.027910	1.02983	29.0	16.30	1.122231	1.12488
8.0	4.53	1.029942	1.03187	29.5	16.57	1.124603	1.12727
8.5	4.82	1.031982	1.03393	30.0	16.85	1.126984	1.12967
9.0	5.10	1.034029	1.03599	30.5	17.12	1.129374	1.13207
9.5	5.38	1.036082	1.03806	31.0	17.40	1.131773	1.13449
10.0	5.67	1.038143	1.04014	31.5	17.68	1.134180	1.13691
10.5	5.95	1.040212	1.04222	32.0	17.95	1.136596	1.13934
11.0	6.23	1.042288	1.04431	32.5	18.23	1.139020	1.14178
11.5	6.51	1.044370	1.04641	33.0	18.50	1.141453	1.14423
12.0	6.79	1.046462	1.04852	33.5	18.78	1.143894	1.14669
12.5	7.08	1.048559	1.05064	34.0	19.05	1.146345	1.14915
13.0	7.36	1.050665	1.05276	34.5	19.33	1.148805	1.15163
13.5	7.64	1.052778	1.05489	35.0	19.60	1.151275	1.15411
14.0	7.92	1.054900	1.05703	35.5	19.87	1.153752	1.15661
14.5	8.20	1.057029	1.05917	36.0	20.15	1.156248	1.15917
15.0	8.48	1.059165	1.06133	36.5	20.42	1.158733	1.16162
15.5	8.76	1.061208	1.06349	37.0	20.70	1.161236	1.16413
16.0	9.04	1.063460	1.06566	37.5	20.97	1.163748	1.16666
16.5	9.33	1.065621	1.06783	38.0	21.24	1.166269	1.16920
17.0	9.61	1.067789	1.07002	38.5	21.51	1.168800	1.17174
17.5	9.89	1.069964	1.07221	39.0	21.79	1.171348	1.17430
18.0	10.17	1.072147	1.07441	39.5	22.06	1.173889	1.17686
18.5	10.45	1.074338	1.07662	40.0	22.33	1.176447	1.17943
19.0	10.73	1.076537	1.07884	40.5	22.60	1.179014	1.18201
19.5	11.01	1.078744	1.08106	41.0	22.87	1.181592	1.18460
20.0	11.29	1.080959	1.08329	41.5	23.15	1.184178	1.18720
20.5	11.57	1.083182	1.08553	42.0	23.42	1.186773	1.18981
21.0	11.85	1.085414	1.08778	42.5	23.69	1.189379	1.19243

° Brix	° Baumé	Specific gravity		° Brix	° Baumé	Specific gravity	
		$\frac{20^{\circ}}{4^{\circ}}$	$\frac{17.5}{17.5}$			$\frac{20^{\circ}}{4^{\circ}}$	$\frac{17.5}{17.5}$
43.0	23.96	1.191993	1.19505	64.5	35.32	1.313304	1.31684
43.5	24.23	1.194616	1.19769	65.0	35.57	1.316334	1.31989
44.0	24.50	1.197247	1.20033	65.5	35.83	1.319374	1.32294
44.5	24.77	1.199890	1.20299	66.0	36.09	1.322425	1.32601
45.0	25.04	1.202540	1.20565	66.5	36.34	1.325484	1.32908
45.5	25.31	1.205200	1.20832	67.0	36.60	1.328554	1.33217
46.0	25.57	1.207870	1.21100	67.5	36.85	1.331633	1.33526
46.5	25.84	1.210549	1.21369	68.0	37.11	1.334722	1.33836
47.0	26.11	1.213238	1.21639	68.5	37.36	1.337821	1.34148
47.5	26.38	1.215936	1.21910	69.0	37.62	1.340928	1.34460
48.0	26.65	1.218643	1.22182	69.5	37.87	1.344046	1.34774
48.5	26.92	1.221360	1.22455	70.0	38.12	1.347174	1.35088
49.0	27.18	1.224086	1.22728	70.5	38.38	1.350311	1.35403
49.5	27.45	1.226823	1.23003	71.0	38.63	1.353456	1.35720
50.0	27.72	1.229567	1.23278	71.5	38.88	1.356612	1.36037
50.5	27.98	1.232322	1.23555	72.0	39.13	1.359778	1.36355
51.0	28.25	1.235085	1.23832	72.5	39.39	1.362953	1.36675
51.5	28.51	1.237859	1.24111	73.0	39.64	1.366139	1.36995
52.0	28.78	1.240641	1.24390	73.5	39.89	1.369333	1.37317
52.5	29.05	1.243433	1.24670	74.0	40.14	1.372536	1.37639
53.0	29.31	1.246234	1.24951	74.5	40.39	1.375749	1.37962
53.5	29.57	1.249046	1.25233	75.0	40.64	1.378971	1.38287
54.0	29.84	1.251866	1.25517	75.5	40.89	1.382203	1.38612
54.5	30.10	1.254697	1.25801	76.0	41.14	1.385446	1.38939
55.0	30.37	1.257535	1.26086	76.5	41.38	1.388696	1.39266
55.5	30.63	1.260385	1.26372	77.0	41.63	1.391956	1.39595
56.0	30.89	1.263243	1.26658	77.5	41.88	1.395226	1.39924
56.5	31.16	1.266112	1.26946	78.0	62.13	1.398505	1.40254
57.0	31.42	1.268989	1.27235	78.5	42.37	1.401793	1.40586
57.5	31.68	1.271877	1.27525	79.0	42.62	1.405091	1.40918
58.0	31.94	1.274774	1.27816	79.5	42.87	1.408398	1.41252
58.5	32.20	1.277680	1.28107	80.0	43.11	1.411715	1.41586
59.0	32.47	1.280595	1.28400	80.5	43.36	1.415040	1.41921
59.5	32.73	1.283521	1.28694	81.0	43.60	1.418374	1.42258
60.0	32.99	1.286456	1.28989	81.5	43.85	1.421719	1.42595
60.5	33.25	1.289401	1.29284	82.0	44.09	1.425072	1.42934
61.0	33.51	1.292354	1.29581	82.5	44.33	1.428435	1.43273
61.5	33.77	1.295318	1.29878	83.0	44.58	1.431807	1.43614
62.0	34.03	1.298291	1.30177	83.5	44.82	1.435188	1.43955
62.5	34.28	1.301274	1.30476	84.0	45.06	1.438579	1.44298
63.0	34.54	1.304267	1.30777	84.5	45.30	1.441980	1.44641
63.5	34.80	1.307271	1.31078	85.0	45.54	1.445388	1.44986
64.0	35.06	1.310282	1.31381	85.5	45.78	1.448806	1.45331

° Brix	° Baumé	Specific gravity		° Brix	° Baumé	Specific gravity	
		$\frac{20^{\circ}}{4^{\circ}}$	$\frac{17.5}{17.5}$			$\frac{20^{\circ}}{4^{\circ}}$	$\frac{17.5}{17.5}$
86.0	46.02	1.452232	1.45678	93.5	49.57	1.504719	1.50996
86.5	46.26	1.455668	1.46026	94.0	49.81	1.508289	1.51359
87.0	46.50	1.459114	1.46374	94.5	50.04	1.511868	1.51722
87.5	46.74	1.462568	1.46794	95.0	50.27	1.515455	1.52087
88.0	46.98	1.466032	1.47074	95.5	50.50	1.519051	1.52449
88.5	47.22	1.469504	1.47426	96.0	50.73	1.522656	1.52810
89.0	47.46	1.472986	1.47778	96.5	50.96	1.526269	1.53180
89.5	47.69	1.476477	1.48132	97.0	51.19	1.529891	1.53550
90.0	47.93	1.479976	1.48486	97.5	51.42	1.533521	1.53920
90.5	48.17	1.483484	1.48842	98.0	51.65	1.537161	1.54290
91.0	48.40	1.487002	1.49190	98.5	51.88	1.540806	1.54665
91.5	48.64	1.490528	1.49556	99.0	52.11	1.544462	1.55040
92.0	48.87	1.494063	1.49915	99.5	52.33	1.548127	1.55413
92.5	49.11	1.497606	1.50274	100.0	52.76	1.551800	1.55785
93.0	49.34	1.501158	1.50635				

73. Calculation for Tenths of a Degree.—The numbers in the other columns corresponding to tenths of a degree Brix when desired can be easily calculated from the table as follows: For Baumé the average difference between 4° and 4.5° Brix is $.28 \div 5 = 0.056$. This number or the corresponding multiple thereof is to be added to the lower or subtracted from the higher Baumé decimal number in the table. Thus if the degree Baumé is desired for the Brix reading 4.3 it is obtained by adding $.056 \times 3 = .168$ to $2.27 = 2.438$ or in numbers of two decimals 2.44 or by subtracting $.056 \times 2 = .112$ from $2.55 = 2.438$ or in places of two decimals 2.44. The specific gravity numbers corresponding to the tenths of a degree Brix are obtained in a similar manner.

In the case cited the specific gravity numbers for $\frac{20^{\circ}}{4^{\circ}}$ and $\frac{17^{\circ}.5}{17^{\circ}.5}$ are as follows:

For $\frac{20^{\circ}}{4^{\circ}}$; $1,983 \div 5 = 397$; $1,013,881 + 397 \times 3$
 $1,015,072$ and $1,015,864 - 397 \times 2 = 1,015,070$.

For $\frac{17^{\circ}.5}{17^{\circ}.5}$; $200 \div 2 = 40$; $1.01570 + 40 \times 3 = 1.01690$
 and $1.01770 - 40 \times 2 = 1.01690$.

Inasmuch as the difference for each whole degree Brix is

variable the calculations must be made with data obtained from the number of each Brix degree.

74. Correction for Temperature.—The following tables show the corrections to be applied to the scale readings when made at any other temperatures, $\frac{17.5^\circ}{17.5^\circ}$, being^a the factor for the first table and $\frac{20^\circ}{4^\circ}$ for the second table.⁸⁰

PER CENT. OF SUGAR IN SOLUTION $\frac{17.5^\circ}{17.5^\circ}$													
Temp.	0	5	10	15	20	25	30	35	40	50	60	70	80
<i>To be subtracted from the degree read.</i>													
0°	0.17	0.30	0.41	0.52	0.62	0.72	0.82	0.92	0.98	1.11	1.22	1.25	1.29
5°	0.23	0.30	0.37	0.44	0.52	0.59	0.65	0.72	0.75	0.80	0.88	0.91	0.94
10°	0.20	0.26	0.29	0.33	0.36	0.39	0.42	0.45	0.48	0.50	0.54	0.58	0.61
11°	0.18	0.23	0.26	0.28	0.31	0.34	0.36	0.39	0.41	0.43	0.47	0.50	0.53
12°	0.16	0.20	0.22	0.24	0.26	0.29	0.31	0.33	0.34	0.36	0.40	0.42	0.46
13°	0.14	0.18	0.19	0.21	0.22	0.24	0.26	0.27	0.28	0.29	0.33	0.35	0.39
14°	0.12	0.15	0.16	0.17	0.18	0.19	0.21	0.22	0.22	0.23	0.26	0.28	0.32
15°	0.09	0.11	0.12	0.14	0.14	0.15	0.16	0.17	0.16	0.17	0.19	0.21	0.25
16°	0.06	0.07	0.08	0.09	0.10	0.10	0.11	0.12	0.12	0.12	0.14	0.16	0.18
17°	0.02	0.02	0.03	0.03	0.03	0.04	0.04	0.04	0.04	0.04	0.05	0.05	0.06
<i>To be added to the degree read.</i>													
18°	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.02
19°	0.06	0.08	0.08	0.09	0.09	0.10	0.10	0.10	0.10	0.10	0.10	0.08	0.06
20°	0.11	0.14	0.15	0.17	0.17	0.18	0.18	0.18	0.19	0.19	0.18	0.15	0.11
21°	0.16	0.20	0.22	0.24	0.24	0.25	0.25	0.25	0.26	0.26	0.25	0.22	0.18
22°	0.21	0.26	0.28	0.31	0.31	0.32	0.32	0.32	0.33	0.34	0.32	0.29	0.25
23°	0.27	0.32	0.35	0.37	0.38	0.39	0.39	0.39	0.40	0.42	0.39	0.36	0.33
24°	0.32	0.38	0.41	0.43	0.44	0.46	0.46	0.47	0.47	0.50	0.46	0.43	0.40
25°	0.37	0.44	0.47	0.49	0.51	0.53	0.54	0.55	0.55	0.58	0.54	0.51	0.48
26°	0.43	0.50	0.54	0.56	0.58	0.60	0.61	0.62	0.62	0.66	0.62	0.58	0.55
27°	0.49	0.57	0.61	0.63	0.65	0.68	0.68	0.69	0.70	0.74	0.70	0.65	0.62
28°	0.56	0.64	0.68	0.70	0.72	0.76	0.76	0.78	0.78	0.82	0.78	0.72	0.70
29°	0.63	0.71	0.75	0.78	0.79	0.84	0.84	0.86	0.86	0.90	0.88	0.80	0.78
30°	0.70	0.78	0.82	0.87	0.87	0.92	0.92	0.94	0.94	0.98	0.94	0.88	0.86
35°	1.10	1.17	1.22	1.24	1.30	1.32	1.33	1.35	1.36	1.39	1.34	1.27	1.25
40°	1.50	1.61	1.67	1.71	1.73	1.79	1.79	1.80	2.82	1.83	1.78	1.69	1.65
50°	—	2.65	2.71	2.74	2.78	2.80	2.80	2.80	2.80	2.79	2.70	2.56	2.51
60°	—	3.87	3.88	3.88	3.88	3.88	3.88	3.88	3.90	3.82	3.70	3.43	3.41
70°	—	—	5.18	5.20	5.14	5.13	5.10	5.08	5.06	4.90	4.72	4.47	4.35
80°	—	—	6.62	6.59	6.54	6.16	6.38	6.30	6.26	6.06	5.82	5.50	5.33

⁸⁰ Bureau of Standards, Density and Volumetric Tables, Circular No. 19: 11.

Temperature in Degrees centi- grade	Observed per cent. of sugar $\frac{20^{\circ}}{4.0^{\circ}}$															
	0	5	10	15	20	25	30	35	40	45	50	55	60	70		
	Subtract from observed per cent.															
0	0.30	0.49	0.65	0.77	0.89	0.99	1.08	1.16	1.24	1.31	1.37	1.41	1.44	1.49		
5	.36	.47	.56	.65	.73	.80	.86	.91	.97	1.01	1.05	1.08	1.10	1.14		
10	.32	.38	.43	.48	.52	.57	.60	.64	.67	.70	.72	.74	.75	.77		
11	.31	.35	.40	.44	.48	.51	.55	.58	.60	.63	.65	.66	.68	.70		
12	.29	.32	.36	.40	.43	.46	.50	.52	.54	.56	.58	.59	.60	.62		
13	.26	.29	.32	.35	.38	.41	.44	.46	.48	.49	.51	.52	.53	.55		
14	.24	.26	.29	.31	.34	.36	.38	.40	.41	.42	.44	.45	.46	.47		
15	.20	.22	.24	.26	.28	.30	.32	.33	.34	.36	.37	.38	.39			
15.56	.18	.20	.22	.24	.26	.28	.29	.30	.30	.32	.33	.33	.34	.34		
(60° F)																
16	.17	.18	.20	.22	.23	.25	.26	.27	.28	.28	.29	.30	.31	.32		
17	.13	.14	.15	.16	.18	.19	.20	.20	.21	.21	.22	.23	.23	.24		
17.5	.11	.12	.12	.14	.15	.16	.16	.17	.17	.18	.18	.19	.19	.20		
18	.09	.10	.10	.11	.12	.13	.13	.14	.14	.14	.15	.15	.15	.16		
19	.05	.05	.05	.06	.06	.06	.07	.07	.07	.07	.08	.08	.08	.08		
	Add to observed per cent.															
21	0.04	0.05	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.09		
22	.10	.10	.11	.12	.12	.13	.14	.14	.15	.15	.16	.16	.16	.16		
23	.16	.16	.17	.17	.19	.20	.21	.21	.22	.23	.24	.24	.24	.24		
24	.21	.22	.23	.24	.26	.27	.28	.29	.30	.31	.32	.32	.32	.32		
25	.27	.28	.30	.31	.32	.34	.35	.36	.38	.38	.39	.39	.40	.39		
26	.33	.34	.36	.37	.40	.40	.42	.44	.46	.47	.47	.48	.48	.48		
27	.40	.41	.42	.44	.46	.48	.50	.52	.54	.54	.55	.56	.56	.56		
27.5	.43	.44	.46	.48	.50	.52	.54	.56	.58	.58	.59	.60	.60	.60		
28	.46	.47	.49	.51	.54	.56	.58	.60	.61	.62	.63	.64	.64	.64		
29	.54	.55	.56	.59	.61	.63	.66	.68	.70	.70	.71	.72	.72	.72		
30	.61	.62	.63	.66	.68	.71	.73	.76	.78	.78	.79	.80	.80	.81		
35	.90	1.01	1.02	1.06	1.10	1.13	1.16	1.18	1.20	1.21	1.22	1.22	1.23	1.22		
40	1.42	1.45	1.47	1.51	1.54	1.57	1.60	1.62	1.64	1.65	1.65	1.65	1.66	1.65		
45	1.91	1.94	1.96	2.00	2.03	2.05	2.07	2.09	2.10	2.10	2.10	2.10	2.10	2.08		
50	2.46	2.48	2.50	2.53	2.56	2.57	2.58	2.59	2.59	2.58	2.58	2.57	2.56	2.52		
55	3.05	3.07	3.09	3.12	3.12	3.12	3.12	3.11	3.10	3.08	3.07	3.05	3.03	2.97		
60	3.69	3.72	3.73	3.73	3.72	3.70	3.67	3.65	3.62	3.60	3.57	3.54	3.50	3.43		

75. Variations Due to Nature of Substance.—The fact that equal per cents. of solid bodies in solution affect the specific

gravity in different degrees has already been noted. The specific gravities of the solutions of the common sugars, however, are so nearly the same for equal per cents. of solid matter in solution as to render the use of a Brix hydrometer quite general for technical purpose. For the mineral salts which often occur in sugar solutions the case is quite different. A 20 per cent. solution of cane-sugar at $17^{\circ}.5$ has a specific gravity of 1.08329 and of dextrose 1.08310, practically identical. But a solution of calcium acetate of similar strength has a specific gravity of 1.0874; of sodium sulfate 1.0807, and of potassium nitrate 1.1359. This latter number would correspond to a sugar content of nearly 27 per cent. The Brix scale can, therefore, be regarded as giving only approximately the percentage of solid matter in sugar solutions and, while useful in technical work, should never be relied upon for exact analytical data.

76. Determination of Density by the Refractometer.—This instrument was first used by Strohmer⁸¹ and developed by Müller.⁸² They showed that the refraction depended on the concentration of the solution. The latter gave a table for converting refractive indices to per cent. sugar. Using the new form of heatable prism of Zeiss, Tolman and Smith found that for equal concentrations⁸³ all sugars have about the same index of refraction.⁸³ They prepared tables for calculating the per cent. of sugar in the solution from the observed index.

Main called attention to the accuracy of this method.⁸⁴ He prepared a table for converting the various readings of the index into per cent. of sugar, when the temperature of observation was 20° .

Geerligs gives the following table for work at 28° and also a table of temperature corrections for the per cent. of sugar obtained when using readings made at other temperatures.⁸⁵

⁸¹ *Zeitschrift für Rübenzucker Industrie*, 1884, 21 : 256 ; 1886, 37 : 91

⁸² *Journal of the American Chemical Society*, 1906, 28 : 1476.

⁸³ *Journal of the American Chemical Society*, 1906, 28 : 1476.

⁸⁴ *International Sugar Journal*, 1907, 9 : 481.

⁸⁵ *International Sugar Journal*, 1908, 10 : 69.

GRERLIGS' TABLE FOR DRY SUBSTANCE IN SUGAR HOUSE PRODUCTS BY
 ABBE'S REFRACTOMETER, AT 28°.

Index	Substance per cent. dry	Decimals	
1.3335	1	0.0001 = 0.05	0.0010 = 0.75
1.3349	2	0.0002 = 0.1	0.0011 = 0.8
1.3364	3	0.0003 = 0.2	0.0012 = 0.8
1.3379	4	0.0004 = 0.25	0.0013 = 0.85
1.3394	5	0.0005 = 0.3	0.0014 = 0.9
1.3409	6	0.0006 = 0.4	0.0015 = 1.0
1.3424	7	0.0007 = 0.5	
1.3439	8	0.0008 = 0.6	
1.3454	9	0.0009 = 0.7	
1.3469	10		
1.3484	11	0.0001 = 0.05	
1.3500	12	0.0002 = 0.1	
1.3516	13	0.0003 = 0.2	
1.3530	14	0.0004 = 0.25	
1.3546	15	0.0005 = 0.3	
1.3562	16	0.0006 = 0.4	
1.3578	17	0.0007 = 0.45	
1.3594	18	0.0008 = 0.5	
1.3611	19	0.0009 = 0.6	
1.3627	20	0.0010 = 0.65	
1.3644	21	0.0011 = 0.7	
1.3661	22	0.0012 = 0.75	
1.3678	23	0.0013 = 0.8	
1.3695	24	0.0014 = 0.85	
1.3712	25	0.0015 = 0.9	
1.3729	26	0.0016 = 0.95	
1.3746	27	0.0001 = 0.05	0.0012 = 0.6
1.3864	28	0.0002 = 0.1	0.0013 = 0.65
1.3782	29	0.0003 = 0.15	0.0014 = 0.7
1.3800	30	0.0004 = 0.2	0.0015 = 0.75
1.3818	31	0.0005 = 0.25	0.0016 = 0.8
1.3736	32	0.0006 = 0.3	0.0017 = 0.85
1.3854	33	0.0007 = 0.35	0.0018 = 0.9
1.3872	34	0.0008 = 0.4	0.0019 = 0.95
1.3890	35	0.0009 = 0.45	0.0020 = 1.0
1.3909	36	0.0010 = 0.5	0.0011 = 1.0
1.3928	37	0.0011 = 0.55	
1.3947	38		
1.3966	39		
1.3984	40		
1.4003	41		

GHERLIGS' TABLE FOR DRY SUBSTANCE IN SUGAR HOUSE PRODUCTS BY
 ABBE'S REFRACTOMETER, AT 28°.—(Continued.)

Index	Substance per cent. dry	Decimals	
1.4023	42	0.0001 = 0.05	0.0012 = 0.6
1.4043	43	0.0002 = 0.1	0.0013 = 0.65
1.4063	44	0.0003 = 0.15	0.0014 = 0.7
1.4083	45	0.0004 = 0.2	0.0015 = 0.75
1.4104	46	0.0005 = 0.25	0.0016 = 0.8
1.4124	47	0.0006 = 0.3	0.0017 = 0.85
1.4145	48	0.0007 = 0.35	0.0018 = 0.9
1.4166	49	0.0008 = 0.4	0.0019 = 0.95
1.4186	50	0.0009 = 0.45	0.0020 = 1.0
1.4207	51	0.0010 = 0.5	0.0021 = 1.0
1.4228	52	0.0011 = 0.55	
1.4249	53		
1.4270	54		
<hr/>			
1.4292	55	0.0001 = 0.05	0.0013 = 0.55
1.4314	56	0.0002 = 0.1	0.0014 = 0.6
1.4337	57	0.0003 = 0.1	0.0015 = 0.65
1.4359	58	0.0004 = 0.15	0.0016 = 0.7
1.4382	59	0.0005 = 0.2	0.0017 = 0.75
1.4405	60	0.0006 = 0.25	0.0018 = 0.8
1.4428	61	0.0007 = 0.3	0.0019 = 0.85
1.4451	62	0.0008 = 0.35	0.0020 = 0.9
1.4474	63	0.0009 = 0.4	0.0021 = 0.9
1.4497	64	0.0010 = 0.45	0.0022 = 0.95
1.4520	65	0.0011 = 0.5	0.0023 = 1.0
1.4543	66	0.0012 = 0.5	0.0024 = 1.0
1.4567	67		
1.4591	68		
1.4615	69		
1.4639	70		
1.4663	71		
1.4687	72		
<hr/>			
1.4711	73	0.0001 = 0.0	0.0015 = 0.55
1.4736	74	0.0002 = 0.05	0.0016 = 0.6
1.4761	75	0.0003 = 0.1	0.0017 = 0.65
1.4786	76	0.0004 = 0.15	0.0018 = 0.65
1.4811	77	0.0005 = 0.2	0.0019 = 0.7
1.4836	78	0.0006 = 0.2	0.0020 = 0.75
1.4862	79	0.0007 = 0.25	0.0021 = 0.8
1.4888	80	0.0008 = 0.3	0.0022 = 0.8
1.4914	81	0.0009 = 0.35	0.0023 = 0.85
1.4940	82	0.0010 = 0.35	0.0024 = 0.9
1.4966	83	0.0011 = 0.4	0.0025 = 0.9
1.4992	84	0.0012 = 0.45	0.0026 = 0.95
1.5019	85	0.0013 = 0.5	0.0027 = 1.0
1.5046	86	0.0014 = 0.5	0.0028 = 1.0
1.5073	87		
1.5100	88		
1.5127	89		
1.5155	90		

TABLE OF CORRECTIONS FOR THE TEMPERATURE

Temperature of the prisms in °C	Dry substance												
	0	5	10	15	20	25	30	40	50	60	70	80	90
20	0.53	0.54	0.55	0.56	0.57	Subtract							0.58
21	0.46	0.47	0.48	0.49	0.50	0.51	0.52	0.54	0.56	0.54	0.53	0.52	0.50
22	0.40	0.41	0.42	0.42	0.43	0.44	0.45	0.47	0.48	0.47	0.46	0.45	0.44
23	0.33	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.39	0.38	0.38	0.38
24	0.26	0.26	0.27	0.28	0.28	0.29	0.30	0.31	0.32	0.31	0.31	0.30	0.30
25	0.20	0.20	0.21	0.21	0.22	0.22	0.23	0.23	0.24	0.23	0.23	0.23	0.22
26	0.12	0.12	0.13	0.14	0.14	0.14	0.15	0.15	0.16	0.16	0.16	0.15	0.14
27	0.07	0.07	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.08	0.07
Add													
29	0.07	0.07	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.08	0.07
30	0.12	0.12	0.13	0.14	0.14	0.14	0.15	0.15	0.16	0.16	0.16	0.15	0.14
31	0.20	0.20	0.21	0.21	0.22	0.22	0.23	0.23	0.24	0.23	0.23	0.23	0.22
32	0.26	0.26	0.27	0.28	0.28	0.29	0.30	0.31	0.32	0.31	0.31	0.30	0.30
33	0.33	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.39	0.38	0.38	0.38
34	0.40	0.41	0.42	0.42	0.43	0.44	0.45	0.47	0.48	0.47	0.46	0.45	0.44
35	0.46	0.47	0.48	0.49	0.50	0.51	0.52	0.54	0.56	0.54	0.53	0.52	0.50

77. Method of Using Geerligs' Table.—In the determination of the density by this method it is only necessary to place a few drops of the solution on the prisms and make a reading, noting the temperature. The per cent. of sugar is then found as given in the following example:

Example.—To find the dry substance in a sugar solution whose refractive index is 1.4589 taken at 26°. The nearest index is 1.4567 which equals 67 per cent. Then 1.4589 minus 1.4567 (the nearest value in the table lower than it) = 0.0022. In the second decimal column to the right look for 0.0022 and the corresponding value is 0.95. The reading is therefore 67.95, but at a temperature of 26° (from the table corrections) 0.16 must be subtracted and the correct per cent. of sugar is 67.79. In like manner the per cent. of sugar of a sample with a refractive index of 1.5021 at 28° would be 85.05, and one of an index of 1.3802 at 28° would be 30.1, and one of an index 1.3655 at 33° would be 22.06.

This method has the advantage over the Areometric and Westphal methods requiring a less quantity of solution for a test, and of giving closer readings to the true per cent. of solids when there are present in solution other substances than sucrose. With pure sucrose solution it is no more accurate than the other methods. Bryan has found that the solids obtained by the refractometer agree very closely with the true solids in maple sirup, cane sirup, honey, commercial glucose and cane and beet molasses.⁸⁰

THE DETERMINATION OF SUGAR WITH POLARIZED LIGHT.

78. Optical Properties of Natural Sugars.—The solutions of all natural sugars have the property of deflecting the plane of polarized light and the degree of deflection corresponds to the quantity of sugar in solution. By measuring the amplitude of the rotation produced the percentage of sugar in the solution can be determined. In order to secure accuracy in the determinations it is necessary that only one kind of sugar be present,

⁸⁰ Journal of the American Chemical Society, 1908, 30 : 1444.

or, if more than one, that the quantities of all but one be determined by other means, and the disturbances produced thereby in the total rotation be properly arranged. In point of fact this process in practice is applied chiefly to sucrose and milk sugars, which occur in nature in an approximately pure state. The process is also useful in determining sucrose, cane-sugar, when mixed with other kinds, by reason of the fact that this sugar after hydrolysis by treatment with a weak acid or invertase for a long time or a strong acid for a short time, definitely changes its rotating power. Since, by the same treatment, the rotating power of most other sugars which may be present is only slightly altered, the total disturbance produced is approximately due to the inversion of the cane-sugar.

Dextrose and maltose arising from the hydrolysis of starch may also be determined with a fair degree of accuracy by their deportment with polarized light. When a solution of natural sugars shows negative results when examined with polarized light, it is due to an admixture of two or more sugars of opposite polarizing powers in such proportions as to produce neutrality. This condition often occurs in the examination of honeys or in submitting artificial sugars to polarimetric observations. In the latter case the neutrality is caused by the tendency manifested by artificially produced sugars to form twin mixtures of optically opposite qualities. In the former case the neutrality may be due to unequal quantities of dextrose and levulose or to the presence of other optical active bodies as dextrin or sucrose.

The instrument used for measuring the degree of deflection produced in a plane of polarized light is called a polariscope, polarimeter, or optical saccharimeter. For theoretical discussion of the principles of polarization and the application of these principles in the construction of polariscopes, the reader is referred to the standard works on optics and the construction of optical instruments.⁸⁷ For the purposes of this work a description of the instruments commonly employed and the methods of using them will be sufficient.

⁸⁷ Landolt, *Handbook for the Polariscope*: 95.

78a. Polarized Light.—When a ray of light has been repeatedly reflected from the bright surfaces or when it passes through certain crystalline bodies it acquires peculiar properties and is said to be polarized.

Polarization is therefore a term applied to a phenomenon of light, in which the vibrations of the ether are supposed to be restricted to a particular form of an ellipse whose axes remain fixed in direction. If the ellipse become a straight line it is called plane polarization. This well-known phenomenon is most easily produced by a nicol prism, consisting of a cut crystal of calcium carbonate (Iceland spar). This rhombohedral crystal, the natural ends of which form angles of 71° and 109° , respectively, with the opposite edges of its principal section, is prepared as follows:

The ends of the crystals are ground until the angles just mentioned become 68° and 112° . The crystal is then divided diagonally at right angles with the planes of the ends and with the principal section, and after the new surfaces are polished they are joined again by Canada balsam. The principal section of this prism passes through the shorter diagonal of the two rhombic ends. If now a ray of light fall on one of the ends of this prism, parallel with the edge of its longer side, it suffers double refraction, and each ray is plane polarized, the one at right angles with the other. That part of the entering ray of light which is most refracted is called the ordinary and the other the extraordinary ray. The refractive index of the film of balsam being intermediate between those of the rays, permits the total reflection of the ordinary ray, which, passing to the blackened sides of the prism, is absorbed. The extraordinary ray passes the film of balsam without deviation and emerges from the prism in a direction parallel with the incident ray, having, however, only half of its luminous intensity.

Two such prisms, properly mounted, furnish the essential parts of a polarizing apparatus. They are called the polarizer and the analyzer, respectively.

If now the plane of vibration in each prism be regarded as co-

incident with its principal section, the following phenomena are observed: If the prisms are so placed that the principal sections lie in the prolongation of the same plane, then the extraordinary polarized ray from the polarizer passes into the analyzer, which practically may be regarded in this position as a continuation of the same prism. The extraordinary polarized ray passes through the analyzer exactly as it did through the polarizer, and is not reflected by the film of balsam, but emerges from the analyzer in seemingly the same condition as from the polarizer. If the analyzer be rotated 180° , bringing the principal section again in the same plane, the same phenomenon is observed. But if the rotation be in either direction only 90° , then the polarized ray from the first prism, incident on the second, departs itself exactly as the ordinary ray, and on meeting the film of balsam is totally reflected. The field of vision, therefore, is perfectly dark.

In all other inclinations of the planes of the principal sections of the two prisms the ray incident in the analyzer is separated into two, an ordinary and extraordinary, varying in luminous intensity in proportion to the square of the cosine of the angle of the two planes.

Thus, by gradually turning the analyzer, the field of vision passes slowly from maximum luminosity to complete obscurity. The expression crossed nicols refers to the latter condition of the field of vision.

79. Description of the Prism.—In a nicol made as described above, Fig. 34 suppose a ray of light parallel with the longer side of the prism be incident to the end $a b$ at m . By the double refracting power of the spar the ray is divided into two, which traverse the first half of the prism. The two rays are polarized at right angles to each other. The less refracted ray when it strikes the film of Canada balsam passes through it without interference. The more refracted ray strikes the balsam at v at such an angle as to be totally reflected and made to pass out of the prism in the direction $o r$. If the prism be blackened at the surface the ray will be entirely absorbed. The other ray passes on through the other half of the prism and emerges in the direction

of qs . It is evident that emergent light from a nicol has only half the illuminating power possessed by the immergent rays.

The polarized plane of light from the nicol just described may

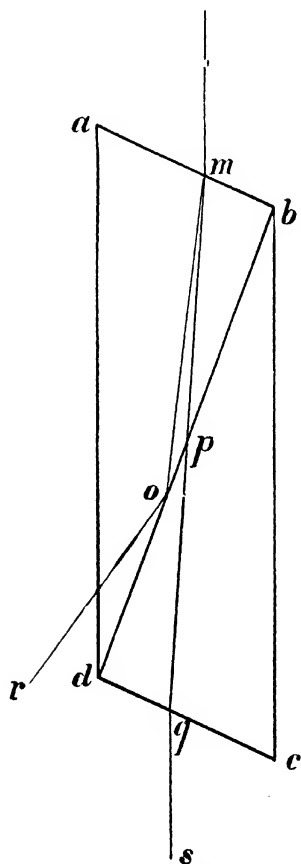


Fig. 34.—Course of Rays of Light in a Nicol.

be regarded as passing also into a second nicol of essentially the same construction as the first.

This second nicol, called the analyzer, is so constructed as to

revolve freely about its longitudinal axis, and is attached to a graduated circle in such a way that the degree of rotation can be accurately read. If the planes of polarization of the two nicols are coincident when prolonged, the ray of light passing from the first nicol will pass through the second practically unchanged in character or intensity. If, however, the analyzing nicol be turned until the plane of polarization is at right angles to that of the polarizer the immergent ray will suffer refraction in such a manner as to be totally reflected when reaching the film of balsam and will be thus entirely lost. In making a complete revolution of the analyzer, therefore, two positions of maximum intensity of light and two of darkness will be observed. In intermediate positions the ray immergent to the analyzer will be separated as in the first instance into two rays of varying intensities, one of which will be always totally reflected.

In respect of the analyzing nicol the following additional observations will be found useful. In all uniaxial crystals there are two directions at right angles to each other, one of greatest and one of least resistance to the propagation of luminous vibrations. These planes are in the direction of the principal axis and at right angles thereto. Only light vibrating in these two directions can be transmitted through calcspar; and all incident light propagated by vibrations in a plane at any other angle to the principal section is resolved into two such component rays. But the velocities of transmission in the two directions are unequal, that is, the refractive index of the spar for the two rays is different. If the analyzing nicol be so adjusted as to receive the emergent light from the polarizer when the corresponding planes of the two prisms are coincident when extended, the emergent extraordinary ray falling into a plane of the same resistance as that it has just left is propagated through the second nicol with the same velocity that it passed in the first one. It is therefore similarly refracted. If, however, the two prisms be so arranged that corresponding planes cross then the extraordinary ray falls into a plane which it traverses with greater velocity than it had before and is accordingly refracted and takes the course which

ends in total reflection at the film of balsam. No light therefore can pass through the prism in that position. If any other substance, as for instance a solution of sugar, capable of rotating a plane of polarized light, be interposed between the two nicols the effect produced is the same as if the analyzer had been turned to a corresponding degree. When the analyzer is turned to that degree the corresponding planes again coincide and the light passes. This is the principle on which the construction of all polarizing instruments is based.⁸⁸

80. The Polariscopes.—A polariscopes for the examination of solutions of sugar consists essentially of a prism for polarizing the light, called a nicol, a tube of definite length for holding the sugar solution, a second nicol made movable on its axis for adjustment to the degree of rotation and a graduated arc for measuring it. Instead of having the second nicol movable, many instruments have an adjusting wedge of quartz of opposite polarizing power to the sugar, by means of which the displacement produced on the polarized plane is corrected. A graduated scale and vernier serve to measure the movement of the wedges and give in certain conditions the desired reading of the percentage of sugar present. Among the multitude of instruments which have been devised for analytical purposes, only a few are found in common use, and the scope of this volume will not allow space for a description of a greater number. For practical discussion of the principles of polarization and their application to optical saccharimetry, the reader may conveniently refer to standard authorities.⁸⁹

81. Kinds of Polariscopes.—For purposes of description three kinds of polarimeters may be mentioned.

1. *Instruments in which the deviation of the plane of polarization is measured by turning the analyzer about its axis.*

Instruments of this kind conform to the simple type first men-

⁸⁸ Landolt, *Handbook for the Polariscopes* : 8.

⁸⁹ Sidersky, *Traité d'Analyse des Matières Sucrées*.

Landolt, *Optical Rotation of Organic Substances* 1902 : 306.

Rolfe, *The Polariscopes in the Chemical Laboratory*, 1905 : 1-14.

tioned, and are *coeteris paribus* the best. The Laurent, Wild, Landolt-Lippich, etc., belong to this class.

2. *Instruments in which both nicols are fixed and the rotation of the plane of polarized light corrected by the interposition of a wedge of a solid polarizing body (quartz).*

Belonging to this class are the apparatus of Soleil, Duboscq, Scheibler, and the compensating apparatus of Schmidt and Haensch, Peters, Fric, etc.

3. *Apparatus in which the analyzer is set at a constant angle with the polarizer, and the compensation secured by varying the length or concentration of the interposed polarizing liquid.*

The apparatus of Trannin belongs to this class.

82. Appearance of Field of Vision.—Polarimeters are also classified in respect of the appearance of the field of vision.

1. *Tint Instruments.*—The field of vision in these instruments in every position of the nicols, except that on which the plane of vibration of the polarized light is coincident with the three principal sections, is composed of two semi-disks of different colors.

2. *Shadow Instruments.*—The field of vision in this class of polarimeters in all except neutral positions, is composed of two or more semi-disks, one dark and one yellow or two dark and one yellow. As the neutral position is approximated the two or more disks gradually assume a light yellow color, and when neutrality is reached they appear to be equally colored. This class includes most of the instruments in use to-day.

3. *Striated Instruments.*—In this class the field of vision is striated. The lines may be tinted as in Wild's polaristrobometer or black, as in the Duboscq and Trannin instruments. The neutral position is indicated either by the disappearance of the striae (Wild) or by the phenomenon of their becoming continuous. (Duboscq, Trannin.)

83. Character of Light Used.—Polariscopes may be further divided into two classes, based on the kind of light employed.

1. *Instruments which Use Ordinary White Light.*—(Oil lamp, etc.) Scheibler, Schmidt and Haensch, Peters, Fric, etc.

2. *Instruments Employing Monochromatic Light.*—(Sodium flame, etc.) Laurent, Landolt-Lippich, etc.

84. Interchangeable Instruments.—Some of the instruments in common use are arranged to be used either with ordinary lamp or gas light, or with monochromatic flame. Laurent's polarimeter is one of this kind. The compensating instruments also may have the field of vision arranged for tints or shadows. Theoretically the best instrument would be one in which the light is purely monochromatic, the field of vision a shadow and the compensation secured by the rotation of the second nicol.

The accuracy of an instrument depends, however, on the skill and care with which it is constructed and used. With quartz wedges properly ground and mounted, and with ordinary white light, polariscopes may be obtained which give readings as accurate as can be desired for analytical purposes.

Since many persons are more or less affected with color blindness, the shadow is to be preferred to the tint fields of vision.

For practical use in sugar analysis the white light is much more convenient than the monochromatic light.

For purposes of general investigation the polarimeters built on the model of the Laurent are to be preferred to all others. Such instruments are not only provided with a scale which shows the percentage of sucrose in a solution, but also with a scale and vernier by means of which the angular rotation which the plane of vibration has suffered, can be accurately measured in more than one-quarter of the circle.

DESCRIPTION OF POLARIZING INSTRUMENTS.

85. Rotation Instruments.—This instrument has already been described as one in which the extent of deviation in the plane of polarized light caused by the intervention of an optically active substance is measured by rotating one of the nicols about its axis and measuring the degree of this rotation by a vernier on a graduated arc.

In Germany these instruments are called *polaristrobometers*, and in France *polarimètres*. In England and this country the

term *polariscope* or *polarimeter* is applied without discrimination to all kinds of optical saccharimeters.

The polariscope of Mitscherlich was one of the earliest in use. It has now been entirely superseded by more modern and accurate instruments.

86. The Laurent Instrument.—A polariscope adapted by Laurent to the use of monochromatic yellow light is almost exclusively used in France and to a considerable extent in this country. In case a worker is confined to the use of a single instrument, the one just mentioned is to be recommended as one well suited to general work. It has the second nicol, called the analyzer, movable and the degree of rotation produced is secured in angular terms directly on a divided circle. The scale is graduated both in angular measurements and in per cents. of sugar for a definite degree of concentration of the solution and length of observation tube. The normal solution in the Laurent instrument contains 16.19 grams of pure sugar in 100 true cubic centimeters, and the length of the observation tube is 200 millimeters. Both the angular rotation and the direct percentage of sugar can be read at the same time. Great accuracy can be secured by making the readings in each of the four quadrants. The light is rendered yellow monochromatic by bringing into the flames of a double bunsen, spoons made of platinum wire, which carry fragments of fused sodium chlorid.

87. The Laurent Burner.—The theory of the illumination of the laurent burner is illustrated by the accompanying Fig. 35. The lamp consists essentially of two bunsens, surmounted by a chimney.⁹⁰ A curved spoon made from platinum gauze serves to hold the fused particles of sodium chlorid which are used to produce the yellow light. The spoon is shown at G, held by the arm F, fastened by the key P. The interior intense flame BB' is surrounded by an exterior less highly colored flame AA. It is important that the optical axis of the polariscope be directed accurately upon the disk B, which is the most intense part of the illumination. The point of the spoon carrying the salt should be

⁹⁰ Sidersky, *Traité d'Analyse des Matières Sucrées*: 104.

coincident with the prolongation of the lamp TT, so that it just strikes the edge of the blue flame. Care should be taken not to press the spoons into the interior of the flame as by so doing the intensity of the illumination is very much diminished. Great care must be observed in the position of the spoon G, and the platinum arm F being flexible, the operator with a little patience, will be enabled to properly place the spoon by bending it. Moreover, if the spoon be pressed too far into the flame, the melted particles of salt collecting in the bottom of G may drop into the lamp and occlude the orifices through which the gas enters. The light of the yellow flame produced by the lamp may be further purified by passing through a prism filled with a solution of

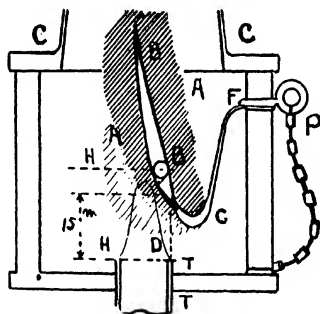


Fig. 35.—Laurent Lamp.

potassium dichromate, or better, a homogeneous disk cut from a crystal of that salt.

Since the flame produced by the above device is not perfectly constant, being more intense at the moment of introducing a fresh portion of the sodium chlorid, Wiley has designed a lamp to supply a constant maximum illumination.

This device which is shown in Fig. 36, is based on the principle of adding constantly a fresh portion of the salt to the flame.⁹¹ The flame is thus kept perfectly uniform in its intensity.

The lamp consists essentially of two wheels with platinum gauze perimeters and platinum wire spokes, driven by a clock-

⁹¹ Journal of the American Chemical Society, 1895, 15: 121.

work D, and mounted by the supports AA' as shown in the figure. The sodium salt, chlorid, bromid or nitrite, in dilute solution, is placed in the porcelain crucibles F, supported by BB' as indicated in the figure, to such a depth that the rims of the platinum wheels dip beneath the surface as they revolve. The salt is volatilized by the lamp E. By means of the crossed bands the wheels are made to revolve in opposite directions as indicated by the arrows. The solution of the salt which is taken up by the platinum net-work of the rim of the wheel, thus has time to become perfectly dry before it enters the flame and the

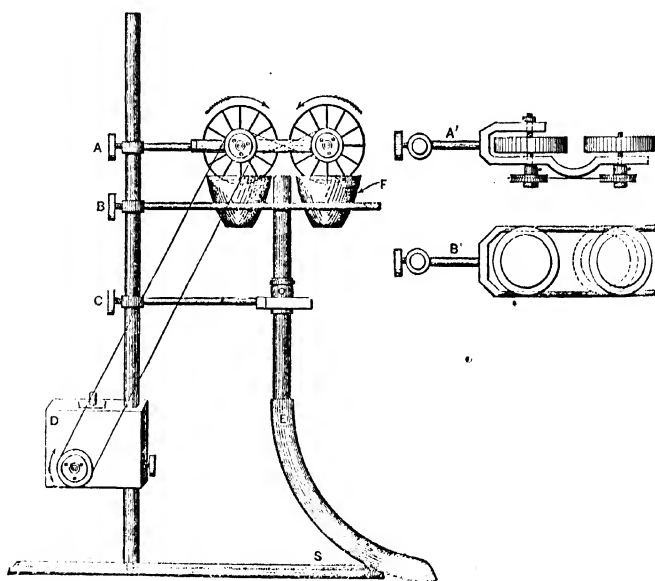


Fig. 36. — Lamp for Producing Constant Monochromatic Flame.

sputtering which a moist salt would produce is avoided. At every instant by this arrangement, a minute fresh portion of salt is introduced into the flame with the result of making a perfectly uniform light which can be used for hours without any perceptible variation. The mechanism of the apparatus is so simple that no further description is necessary. The polariscope

should be so directed toward the flame as to bring into the field of vision its most luminous part. The platinum wheels are adjustable and should be so arranged as to produce between them an unbroken yellow flame. The wheels are eight centimeters in diameter and are driven at a rate to make one revolution in from six to ten minutes.

88. Construction of Laurent's Apparatus.—The shadow polariscope invented by Laurent is constructed as follows: The polarizer is a special nicol which is not fixed in its position, but is so arranged as to be turned through a small arc about its axis. With each change of the polarizer, the analyzer must be reset, so that the zero point of the scale will be the neutral field. By this device, the quantity of light passing through it can be regulated, and the apparatus is thus useful with colored solu-

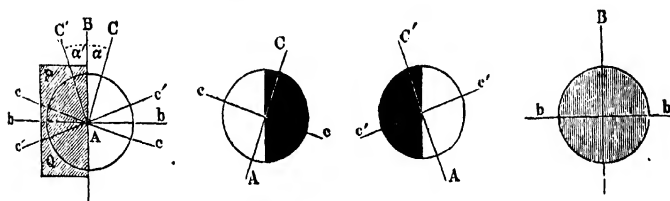


Fig. 37. *Field of Vision of a Laurent Polariscope.

tions which are not easily cleared by any of the common bleaching agents. The greater the quantity of light admitted, however, the less delicate is the reading of the shadow produced. The plane of polarized light emergent from this prism, falls on a disk of glass half covered by a thin lamina of quartz which thus divides the field of vision into halves. It is this semi-disk of quartz which is the distinguishing feature of the apparatus. The polarized light thus passes without hindrance the half field of vision which is covered by the glass only, but can not pass the quartz plate unless its axis is set in a certain way. The field of vision may be thus half dark, or both halves may be equally illuminated or equally dark according to the position of the nicol analyzer which is freely movable about its axis and carries a vernier and reading glass over graduated

circle. The field of vision in the laurent may have any of the following forms (Fig. 37).⁹² Let the polarizer be first so adjusted that the plane of polarization of the transmitted pencil of light is parallel to the axis of the plate lying in the direction A B. The two halves of the field of vision will then appear equally illuminated in every position of the analyzer. But if the polarizing nicol be inclined to AB at an angle α , the plane of polarization of the rays passing through the quartz plate will undergo deviation through an equal angle in the opposite direction.

It happens from this, that when in the uncovered half of the field, the plane of polarization has the direction AC, in the other half it will have the direction AC'. When the analyzer is rotated, if this plane of polarization lie in the direction cc, the rays polarized parallel to AC will be completely extinguished and the corresponding half of the field will be dark. The opposite happens when the plane of polarization lies in the direction of c' c'. When one half of the field is thus obscured, the other suffers only a partial diminution in the intensity of its illumination. When the middle position b b is reached in the rotation of the analyzer, the illumination of the two halves is uniform, and this is the point at which the zero of the scale is reached. The slightest rotation of the analyzer to the right or left of this neutral point will cause a shadow to appear on one of the halves of the field, which by an oscillatory movement of the analyzer, seems to leap from side to side. The smaller the angle α or BAC, the more delicate will be the shading and the more accurate the observation. This angle being adjustable by the mechanism already described, should be made as small as will permit the admission of the quantity of light requisite for accurate observation.

The various pieces composing the polariscope are arranged in the following positions, beginning on the right of Fig. 38 and passing to the left, where the observer is seated.⁹³

⁹² Landolt, Handbook of the Polariscope : 120.

⁹³ Sidersky, Traité d'Analyse des Matières Sucrées : 97.

1. The lamp VV, TT, AA, or the wheel burner:
2. The lens B for condensing the rays and rendering them parallel:
3. The tube I, blackened inside to carry the lens:
4. A thin lamina E, cut from a crystal of potassium bichromate, serving to render the sodium light more monochromatic: When the saccharine liquids under examination are colored the crystal of bichromate is removed before the observation is made.
5. The polarizer R, which is rotatable through a small angle by the lever K:

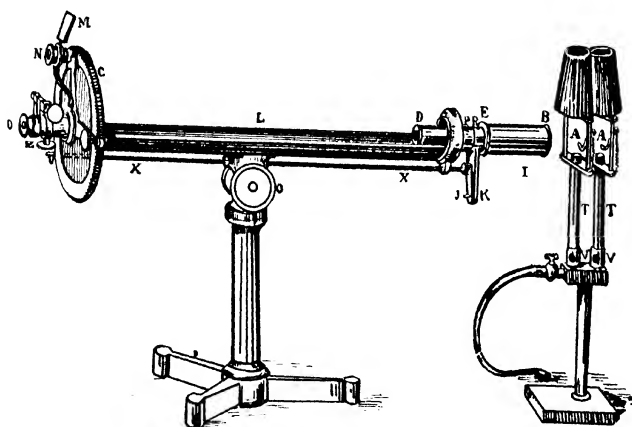


Fig. 38.—Laurent Polariscopes.

6. The lever JK for rotating the tube containing the polarizer: This is operated by the rod X extending to the left.
7. Diaphragm D, half covered with a lamina of quartz.
8. Trough L for holding the observation tube: In the large instrument shown in the figure, it is more than half a meter in length and arranged to hold an observation tube 500 millimeters long.
9. Disk C, carrying divided circle and arbitrary sugar scale:
10. Mirror M, to throw the light of the lamp on the vernier of the scale:

11. Reading glass N, carried on the same radius as the mirror and used to magnify and read the scale:

12. Device under N, to regulate the zero of the instrument:

13. Tube carrying a nicol analyzer and ocular O for defining the field of vision: This tube is rotated by the radial arm carrying the mirror and reading glass.

89. Manipulation.—The lamp having been adjusted, the instrument, in a dark room, is so directed that the most luminous spot of the flame is the line of vision. An observation tube filled with water is placed in the trough and the zero of the vernier is placed accurately on the zero of the scale. The even tint of the field of vision is then secured by adjusting the apparatus by the device, number 12.

90. The Soleil-Ventzke Polariscopes.—A form of polariscope giving a colored field of vision was in use in this country almost exclusively until within 25 years, and may be still employed in some laboratories. There are many forms of tint instruments, but the one almost exclusively used in this country is that mentioned. A full description of their construction and manipulation is given by Tucker.⁹⁴ By the introduction of a third rotating nicol in front of the lens next to the lamp, the sensitive tint at which the reading is made can be kept at a maximum delicacy. These instruments are capable of rendering very reliable service, especially in the hands of those who have a delicate perception of color. They are inferior, however, to the shadow instruments in delicacy, and are more trying to the eye of the observer. The shadow instruments therefore, especially those making use of an ordinary kerosene lamp, have practically driven the tint polariscopes out of use.

The general arrangement of a tint instrument as modified by Scheibler is shown in Fig. 39.

Beginning on the right of the figures, its optical parts are as follows: A is a nicol which, with the quartz plate B, forms the apparatus for producing the light rose neutral tint. The proper degree of rotation of these two parts is secured by means of the

⁹⁴ Manual of Sugar Analysis : 143.

button L attached to the rod carrying the ratchet wheel as shown. The polarizing nicol is at C, and D is a quartz disk, one-half of which is right-handed and the other left-handed. At G is another quartz plate belonging to the analyzing part of the apparatus. This, together with the fixed quartz wedge F, and the movable quartz wedge E, constitute the compensating apparatus of the instrument whereby the deviation produced in the plane of polarized light by the solution in the tube is measured.

Next to the compensating apparatus is the analyzing nicol which in this instrument is fixed in a certain place, viz., the zero of the scale. The analyzer and the telescope for observing

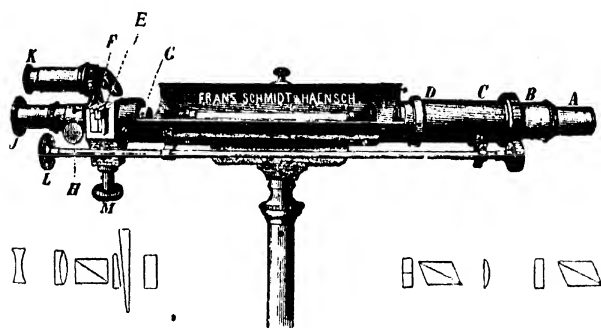


Fig. 39.—Tint Polariscopes.

the field of vision are carried in the tube HJ. The movable quartz wedge has a scale which is read with a telescope K, provided with a mirror inclined at an angle of 45° , just over the scale and serving to illuminate it. The quartz wedges are also provided with a movement by which the zero point of the scale can be adjusted. A kerosene lamp with two flat wicks is the best source of illumination and the instrument should be used in a dark room and the light of the lamp, save that which passes through the polariscopes, be suppressed by a shade. The sensitive or transition tint is produced by that position of the regulating apparatus which gives a field of view of such a nature that a given small movement of the quartz compensating wedge

gives the greatest contrast in color between the halves of the field of vision. For most eyes a faint rose-purple tint, as nearly colorless as possible, possesses this quality. A slight movement of the quartz wedge by means of the screw head M will, with this tint, produce on one side a faint green and on the other a pink color, which are in strong contrast. The neutral point is reached by so adjusting the quartz wedge as to give to both halves of the field the same faint rose-purple tint.

91. The Shadow Polariscope for Lamp Light.—This form of instrument is now in general use for saccharimetric purposes. It possesses on the one hand, the advantages of those instruments using monochromatic light, and on the other, the ease of manip-

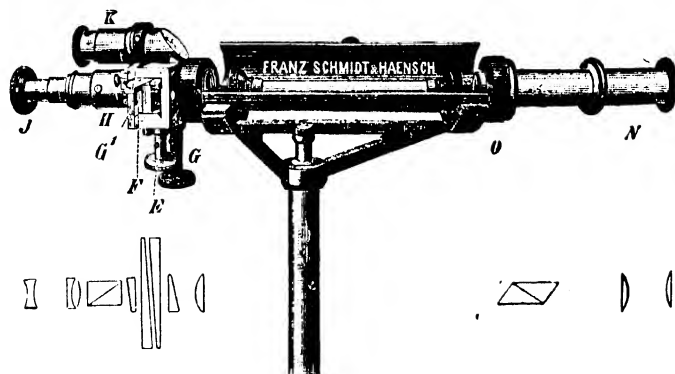


Fig. 40. Double Compensating Shadow Polariscope.

ulation possessed by the tint polariscopes. It differs from the tint instrument in dispensing with the nicol and quartz plate used to regulate the sensitive tint, and in having its polarizing nicol of the Jellet-Cornu type, or having the Lippich polarizer, which consists of the main nicol and a small nicol, placed in front for the half shade. The more improved forms of the apparatus have a double quartz wedge compensation. The two wedges are of opposite optical properties, and serve to make the observations more accurate by mutual correction. The optical arrangement of the different parts of such a polariscope is shown in Fig. 40.

The lenses for concentrating the rays of light and rendering them parallel are contained in the tube N. At O is placed the modified polarizing nicol. The two compensating quartz wedges are moved by the milled screw-heads EG. The rest of the optical apparatus is arranged as described under the tint polariscope. For practical purposes, only one of the wedges is employed, but for all accurate work the readings should be made with both wedges and thus every possible source of error eliminated.⁹⁵

92. The Triple Field Instrument.—When properly made, all the instruments which have been mentioned, are capable of

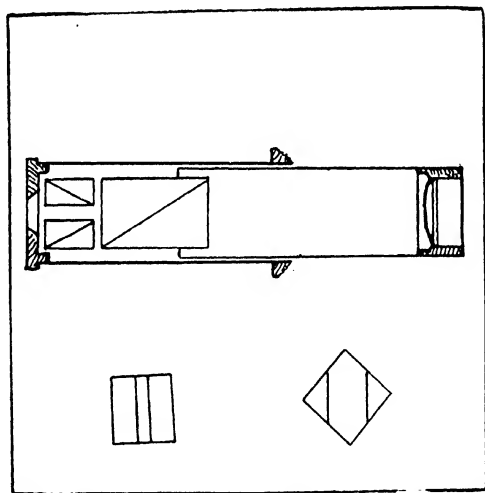


Fig. 41. Apparatus for Producing a Triple Field.

giving accurate results if used in harmony with the directions given. In the use of polariscopes having colored fields of vision a delicate sense of distinguishing between related tints is necessary to good work. Color-blind observers could not successfully use such apparatus. In the shadow instruments it is only necessary to distinguish between the halves of a field of vision unequally illuminated and to reduce this inequality to zero. A

⁹⁵ Landolt, *Optical Rotation of Organic Substances* : 335.

neutral field is thus secured of one intensity of illumination and of only one color, usually yellow. Such a field of vision permits of the easy discrimination between the intensity of the coloration of its two halves, and is consequently not trying to the eye of the observer, and allows of great accuracy of discrimination. This field of vision has lately been still further modified by dividing it into three parts instead of two. A quadruple field of vision has also been devised by Lummer but it has but little practical interest.

The triple field is secured by interposing in front of the polarizing nicol two small nicols as indicated in Fig. 41 The

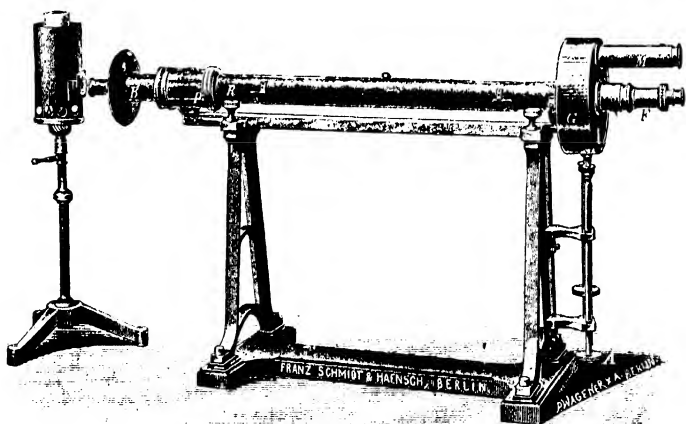


Fig. 42. Polariscope Showing Protected Parts.

end views in different positions of the polarizer are shown in the lower part of the diagrams.

Instead of the comparison of the intensity of the illumination being made on the halves of the field of vision it is made by comparing the segments of the halves with a central band, which also changes in intensity synchronously with the two segments, but in opposite direction. Experience fails to show any marked superiority of the triple over the double field.

Recent improvements in this form of instruments includes an

air-tight boxing of the parts used in reading the scale and in etching the scale on glass. This device is illustrated in Fig. 42.

A metal disk near the lamp shields the source of light from the observer. It has a lens placed at the opening to conduct some of the rays to the scale situated in G. At B is situated the light filter cell. P is the polarizer which may be any one of the many mentioned. If a Lippich polarizer is employed the angle of shadow is not capable of being changed. At G enclosed in a dust-proof box is the quartz wedge compensation operated by

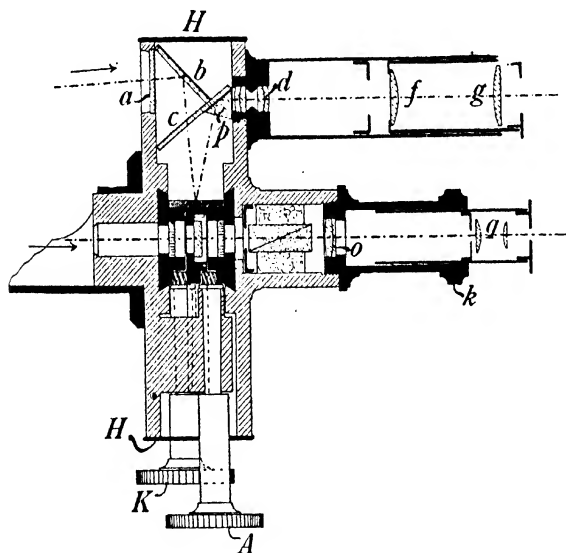


Fig. 43.—Diagram of Arrangement of Reading Apparatus.

the long and short lever at A. F is the telescopic arrangement for focusing the field of vision. M is also a telescopic arrangement for reading the scales and verniers.

The arrangement for reading the scales in g is shown in Fig. 43, showing a longitudinal cross section of the parts, the method of lighting the scale; and the quartz wedge arrangement in place. The light enters from the source described above in the direction of the arrow.

93. Peters Instrument.—A somewhat different type of polariscope known as Peters has lately come into use. Its general

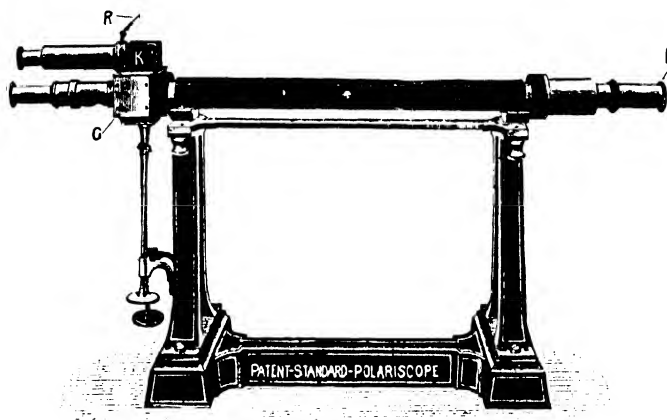


Fig. 44 Peter's Polariscope.

form is shown in Fig 44. The optical parts are practically the same as the previous ones, except in the lighting of the scale, the mirror taking the light and reflecting it on the enclosed scale of the wedges.



Fig. 45.

The optical parts are shown in detail in Fig. 45. The polarizing apparatus is the Lippich half shade $N_1 N_2$. The double compensation quartz wedges are shown in front of the analyzer N_3 .

94. Fric Polariscope.—The Fric instrument has the latest improvements including the glass scales. With this type of scale the reading of the vernier is easier and it is possible to estimate accurately hundredths of a per cent.

The control scale is reddish in color, so as not to be confused with the other scale.

95. Bates' Modification.—In all the direct reading polariscopes described, there is no way of changing the sensitiveness of the instrument, that is, changing the half shade angle.⁹⁵ It has been previously noted that when the Lippich polarizing apparatus is

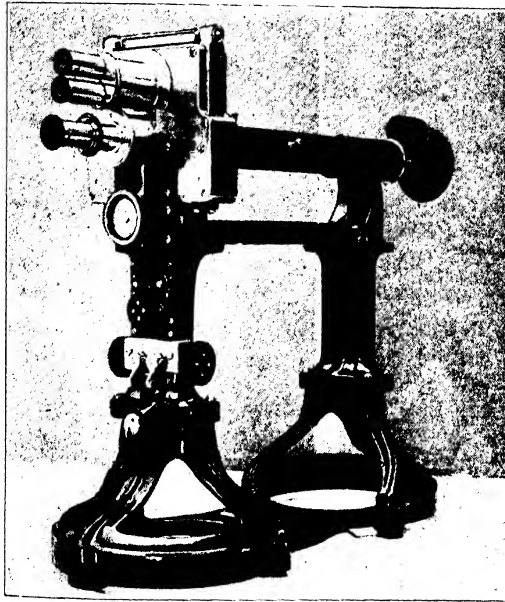


Fig. 46.—Bates' Polariscopes.

used in this type of instrument the large prism is not rotated and no change of angle is possible. Bates has modified the apparatus by providing a mechanism whereby both analyzer and polarizer are rotated simultaneously through the correct angles to give any desired sensibility and brightness without material change of the zero point or other correction. The half shadow

⁹⁵ Bulletin Bureau of Standards, 4 : 461, (Reprint No. 86).

angle in the latest models is capable of adjustment through 4° to 15° .

The polariscope as modified by Bates is shown in Fig. 46. Many points are similar to the last described instrument, as the lighting of the vernier, the two reading tubes for the scales, and the quartz wedges. The keys for moving the wedges are on the side of the frame with suitable locks shown in front. The instrument is provided with a thermometer extending down near the wedges, so that their temperature can be ascertained.

The Boch stand is much heavier than in the other instruments, giving it stability. Schönrock has criticised the mathematical data involved in the discussion of the instrument.⁹⁷

Bates⁹⁸ in reply to this criticism holds that his calculations are correct. This instrument is to be installed at all Port laboratories of the United States Custom Service.

96. Landolt Lippich Instrument.—The construction of most polariscopes is such that tubes of not over a certain diameter can be used to hold the solution to be polarized. In ordinary construction the polarizer is placed at one end of a hollow trough and the analyzer at the other, various lenses being also intervened to make readings easier in part and to bring the field in view. Landolt has designed an apparatus for more general use where not only tubes of varying sizes may be brought between the polarizer and analyzer but vessels of irregular shape may be interposed also where the distance between the analyzer and the polarizer may be shortened or lengthened, as the exigency of the occasion may direct.⁹⁹ This is accomplished by a separate mounting for the analyzer and polarizer and for the vessel containing the tube or the tube itself. These separate mountings are capable of being clamped on a rigid frame and their height above this is capable of adjustment.

A special form of this apparatus for exact measurements is shown in Fig. 47. The cut is made from the apparatus supplied

⁹⁷ *Zeitschrift des Vereins der Zucherindustrie*, 1908, **58** : III-4.

⁹⁸ *Bulletin Bureau of Standards*, **5** : 193, (Reprint No. 98).

⁹⁹ *Berichte der deutschen Chemischen Gesellschaft*, **28** : 3102.

by Schmidt and Haensch for the Bureau of Chemistry.¹ Measurements are capable of being made to 0.001 with a mean error

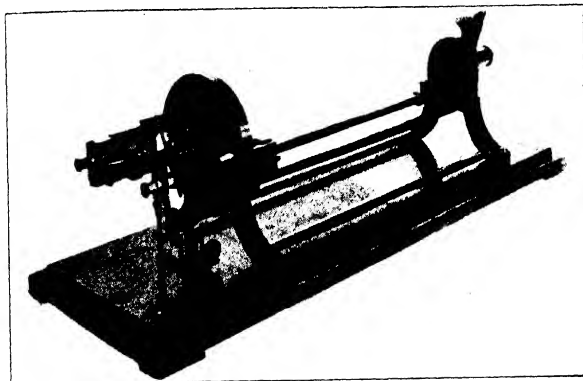


Fig. 47.—Landolt-Lippich Polariscopes.

according to Landolt of 15" or about 0.004. For this very exact work, special light filters are used and monochromatic light.

THE ANALYTICAL PROCESS.

97. General Principles.—Having described the instruments chiefly employed in the optical examination of sugar solutions, the next step is to apply them to the analytical work. A common set of directions for use will be found applicable to all instruments with such modifications only as are required by peculiarities of construction. With the best made instruments it is always advisable to have some method of controlling the accuracy of construction. The simplest way of doing this is to test the apparatus by standard quartz plates. These plates, made from quartz crystal ground to definite thickness, may be either levo or dextro rotary and have been accurately tested by standard instruments. Theoretically such quartz plates deflect the plane of polarized light in a degree proportionate to their thickness, but practically some small deviations from the rule are found. With

¹ Landolt, 2d Edition : 361.

a source of light of the same tint, and at a constant temperature, such plates become a safe test for the accuracy of the graduation of polariscopes. They are more convenient for use than pure sugar solutions of known strength which are the final standards in all disputed cases. These quartz plates are conveniently mounted in tubes of the same size as those holding the sugar solution, and thus fit accurately into the trough of the polariscope, the optical axis of which passes through their center. The quartz plate when used for setting the scale of a polariscope should be placed always in the same position. In some plates slight differences of reading may be noticed on rotating the tubes holding them. Theoretically, such differences should not exist, but in practice they are sometimes found. The temperature of observation should also be noted, and if not that at which the value of the plate was fixed, a proper correction should be made. Care must be taken that the quartz plates in the tubes are not subjected to pressure. They are best held lightly by means of wax with just enough pressure to keep them from moving.

98. Setting the Polariscope.—The following description of setting the polariscope is especially adapted to a shadow instrument, but the principles of adjustment are equally applicable to all.

The scale of the instrument is first so adjusted by means of the adjusting screws provided on each instrument, as to bring the zero of the vernier and that of the scale exactly together. The telescope or ocular is then adjusted until the sharp line separating the halves of the field of vision is brought into focus. This being accomplished an observation tube filled with pure water is placed in the apparatus and the telescope again adjusted to bring the dividing line of the field into focus. The beginner especially, should repeatedly study this adjustment and be impressed with the fact that only in a sharply defined field are practical observations of any worth. The importance of having all the lenses perfect and all the cover glasses without a flaw may be fully appreciated when it is remembered that the polarized ray, already deprived of half its original luminous power, must pass through several centimeters of crystallized calcium

carbonate, and half a dozen disks of glass and quartz, and as many lenses before reaching the eye of the observer. The end covers of the observation tubes may also show optical activity especially if subjected to pressure from the caps. Only with the greatest care and neatness is it possible to secure the required degree of illumination. The zero point having been well studied and accurately adjusted, the scale of the instrument may be tried with a series of quartz plates of known polarizing power at the temperature of the observation. In the apparatus with double quartz wedge compensation, it will be noticed that the marks on one scale are black and on the other red. The black is the working and the red the control scale. To operate this instrument, the red scale is placed exactly at the zero point. The black scale is also placed at zero, and if the field of vision is not neutral, it is made so by the micrometer screw with which the black scale is provided. This should only be used when a series of readings have shown the zero to be wrong. When more than one person is reading the instrument a correction for the zero should be used instead of changing it for each observer by this means. In a right-handed solution, the red scale is left at zero and the black one moved to the right until neutrality in the field of vision is reached and the reading is taken. The observation tube containing the sugar solution is taken out and the red scale moved until the field of vision is again neutral and the reading of the red scale taken. The two readings should agree. Any failure in the agreement shows some fault either in adjusting the apparatus or in its construction, or some error in manipulation.

The double compensating shadow instruments are more readily tested for accuracy in all parts of the scale than those of any other construction. The two compensating wedges are cut with the greatest care, one from a left-handed and the other from a right-handed perfectly homogenous quartz crystal. Since faults in these wedges are due either to lack of parallelism of surface, or of perpendicularity to the optical axis of the crystal, and since these faults of crystallization or construction must be in a very limited degree common they would not coincide once in many

thousand times in the two wedges. This is easily shown by the theory of probabilities. If, therefore, the two readings made at any point, should not agree, it must be due either to a fault in one of the wedges, or to a fault in reading or a lack of adjustment, as has been mentioned. In such cases the readings should be retaken and the errors are usually easily discovered. The lamp is placed about six inches from the end of the instrument and screened so that no light is seen except the bundle of rays falling on the lens.

99. Light Filter Cells.—When using white light, there is generally a predominance of blue rays which greatly disturb the matching of the colors of the two shades in the half shadow instruments. To do away with this, the light to be polarized should be passed through a solution of potassium bichromate. Most instruments are provided with cell for this purpose in the polarizing end. The best strength of solution to use is three per cent. when using a three centimeter cell or six per cent. in a one and a half centimeter cell. Other instruments have a bichromate crystal in the ocular to accomplish the same purpose. Where highly colored sugar solutions are polarized this cell is not necessary, but it is of great importance in light colored solutions.

It is best to have the source of light in a different room and this is essential when the reading room is to be kept at a constant temperature.

100. Setting the Polariscopes with Quartz Plates.—Pure sugar is not always at the command of the analyst, and it is more convenient practically to adjust the instrument by means of quartz plates, the sugar values of which have been previously tested for the character of the light used. Assuming the homogeneity of a plate of quartz, the degree of deflection which it imparts to a plane of polarized light depends on the quality of the light, the thickness of the plate, and the temperature.

In respect of the quality of light, red polarized rays are least, and violet most deflected. The degree of rotation produced with any ray, at a given temperature, is directly proportional to the thickness of the plate. Temperature affects the rotating

power of a quartz plate in a degree highly significant from a scientific point of view and not wholly negligible for practical purposes. The rotating power of a quartz plate increases with the temperature and the variation may be determined by the formula given below.²

The formula is applicable for temperatures between 0° and 100°. Its values are expressed in degrees of angular measure which can be converted into degrees of the sugar scale by appropriate factors:

Formula.— $a^t = a^0 (1 + 0.0001469t)$; in which a^0 = polarization in angular degrees at 0°, t the temperature of observation and a^t the rotation desired.

Example.—A quartz plate which has an angular rotation of 33° at 0° will have a rotation at 20° of 33°.09834.

$$a^t = 33(1 + [0.000149]20) = 33.09834.$$

Since in instruments using the ventzke scale one degree of the sugar scale is equal to 0.3467 degree angular measure, the sugar value of the quartz plate mentioned is equal to 95.47 per cent.; $33.09834 \div 0.3467 = 95.47$.

The sugar value of this plate at 0° is $33 \div 0.3467 = 95.18$.

At the present time the value of quartz plates is ascertained at a temperature of 20° for monochromatic yellow light (D line of the spectrum). In these conditions the true value for degrees ventzke is ascertained by the factor 0.34657.³ $100^\circ V = 34^\circ.657$ circular measure.

101. Measurements by Bureau of Standards.—The Bureau of Standards measures plates for control purposes in circular degrees at 20° C. The rotation is given either for the wave-length of 589.25 $\mu\mu$ or 546.1 $\mu\mu$ as desired. These wave-lengths represent the two sodium lines of incandescent sodium vapor and the yellow green line of incandescent mercury vapor respectively. If no choice is indicated the measurement will be for the sodium lines.⁴

102. Applicability of Quartz Plates.—Quartz plates which are correctly set for one instrument or kind of light, should be equally

² Landolt and Börnstein, *Physikalisch-Chemische Tabellen*, 1905: 707.

³ *Zeitschrift des Vereins der deutschen Zucker-Industrie*, 1909, 59: 523.

⁴ Bureau of Standards, Circular 12, 1906: 2.

accurate for the sugar scales of all instruments, using the same sugar factor. In other words a quartz plate which reads 99° on a Scheibler color polariscope, should give the same reading on the sugar scale of a shadow compensating or a monochromatic direct reading apparatus using 26.048 grams of sugar.

The most useful quartz plates for sugar analysis, are those which give the readings at points between 80° and 96° , which cover the limits of ordinary commercial sugars. For molasses the plates should read from 45° to 55° . For sugar juices of the cane and beet, the most convenient graduation would be from 10° to 20° , but plates of this value would be too thin for practical work and are not in use. Combinations of left-handed and right-handed plates may be used for such low graduations. For instance a right-handed plate reading 98° combined with a left-handed plate reading 88° could be used to test the $+ 10^\circ$ part of the scale. When quartz plates are to be used for control purposes, they should be purchased from reliable manufacturers, and also tested directly against pure sugar solutions by the observer.

Quartz plates as a rule, are true to their markings.

PREPARATION OF PURE SUCROSE AND ITS USE.

103. Control Observation Tube.—Instead of using quartz plates of known values for testing the accuracy of the scale, an observation tube may be used, the length of which can be varied at the pleasure of the observer.

The construction of a tube of this kind is shown in Fig. 48. The tube B is movable in A by means of the ratchet wheel shown. It is closed at D water-tight by a glass disk. The tube B fits as accurately into A as is possible to permit of free movement, and any liquid which may infiltrate between its outer surface and the inner surface of A is prevented from gaining exit by the washer, C, which fits both tubes water tight. The ratchet which moves B in A carries a millimeter scale and vernier X whereby the exact thickness of the liquid solution between the surfaces of the glass disks D and E can be always determined.

By this device the length of liquid under observation can be

accurately read to a tenth of a millimeter. The cover glass E is held in position by any one of the devices in common use for this purpose in the case in question, by a bayonet fastening. The funnel T, communicating directly with the interior of A, serves to hold the solution, there being always enough of it to fill the tube when D is removed to the maximum distance from C, which is usually a little more than 200 millimeters.

Let the control tube be adjusted to 200 millimeters and filled with a solution of pure sugar, which reads 100 per cent. or degrees in a 200 millimeter tube. Since the degree of rotation is, other things being equal, proportional to the length of the column of polarizing solution, it follows that if the tube B be removed inward until the distance between D and C is 100 millimeters, the

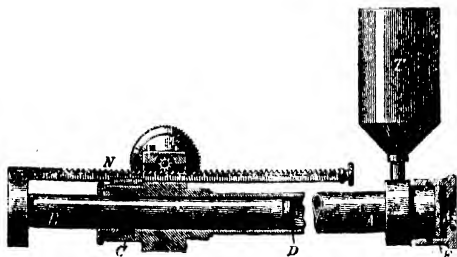


Fig 48. Control Observation Tube.

scale should read 50° or per cent. By adjusting the length of the distance between B and C it is easily seen that every part of the scale can be accurately tested.

The tube should be filled by removing the funnel and closing the orifice with a screw cap which comes with the apparatus. The cap E is then removed and the tube filled in the ordinary manner. This precaution is practiced to avoid carrying air bubbles into the tube when filled directly through the funnel. With a little care, however, this danger may be avoided, or should air bubbles enter they can be easily removed by inclining the tube.

In case the solution used be not strictly pure it may still be employed for testing the scale. Suppose, for instance, that a

solution made up in the usual way, has been made from a sample containing only 99.4 per cent. of sugar. Then in order to have this solution read 100° on the scale the tube should be set

at 201.2 millimeters, according to the formula $\frac{200 \times 100}{99.4} = 201.2$.

By a similar calculation the position of the tube for reading any desired degree on the scale can be determined. The importance of controlling all parts of the scale in compensating instruments is emphasized by the fact that a variation of only 0.016 millimeter in the thickness of the compensating wedge will cause a change of one degree in the reading of the instrument.

104. The Sugar Flask.—For sugar work the true 100 gram flask is not usually employed, but one graduated by weighing at 17°.5 has heretofore been commonly used.⁵ At the present time 20° has been generally accepted as the proper temperature for graduation. These flasks are graduated by first weighing them perfectly dry, filling with distilled water and again weighing 50 and 55, or 100 and 110 grams of water at the temperature named. Since the volume of water at 17°.5 is greater than at 4° the sugar flask in ordinary use has a greater volume by about 0.25 cubic centimeter than the true flask. The observer should always secure a statement from the dealer in respect of the volume of the flask used in testing the scale of the polariscope purchased. One hundred cubic centimeters of water measured at 17°.5 becomes 100.0490 cubic centimeters at 20°, 100.1634 at 25° and 100.229 at 27°.5. In the graduation of a flask in true cubic centimeters, when brass weights are used it will be necessary to correct the weight of each gram of water by adding to it one milligram, which is almost exactly the weight of the volume of air displaced by one gram of water in the circumstances named. If the flask be first counterbalanced and it be desired to mark it at 100 cubic centimeters the sum of the weights placed in the opposite pan should be $100 - 0.100 = 99.900$ grams. While this is not a rigidly exact correction it will be sufficient for all practical purposes. A liter of dry air weighs 1.29366 grams; and

⁵ The methods for standardizing flasks used by the Bureau of Standards are given by Osborn, in Bureau of Standards, Bulletin 4, 1908 : 554.

100 cubic centimeters of water would therefore displace 0.129 gram of air. But the brass weights also displace a volume of air which when deducted reduces the correction to be made for the water to nearly the one named. For convenience in inverting sugar solutions the flasks used in practical work are graduated at 50 and 55 and 100 and 110 cubic centimeters respectively.

105. Normal Sugar Solution.—A normal sugar solution may be prepared in the flasks ordinarily in possession of the analyst. If 26.048 grams of sugar be weighed in air with brass weights, dissolved and made up to the 100 cubic centimeter mark on a Mohr flask, (100.234 true cubic centimeters) it will have a specific gravity of 1.100⁶ at $\frac{17^{\circ}.5}{17^{\circ}.5}$. This solution contains 23.685 per cent. by weight of sugar.⁷ Or 26 grams of sugar may be used in a true 100 cubic centimeter flask.

Preparation of pure sugar: The following method of purifying sugar for use in testing polariscopes, was adopted by the Congress of Applied Chemistry, Paris, 1900:

Prepare a hot saturated solution of the purest commercial sugar obtainable, and precipitate the sugar with absolute alcohol. Spin the precipitated sugar in a small centrifugal and wash it with alcohol. Redissolve, reprecipitate, and wash the sugar as above. The sugar is dried between pieces of blotting paper and preserved in a stoppered jar. The moisture in the sugar should be determined and proper allowance made for it when weighing the sample for analysis.

106. Preparing Sugar Solutions for Polarization.—If sugar samples were always pure the percentage of sugar in a given solution could be directly determined by immediate polarization. Such cases, however, are rarely met in practice. In the majority of cases the sample is not only to be brought into solution but is also to be decolorized and rendered limpid by some one of the methods to be described. A perfectly limpid liquid is of the highest importance to secure correct observations. With a cloudy

⁶ Landolt, Das optische Drehungsvermögen : 334.

⁷ Zeitschrift des Vereins für die Rübenzucker-Industrie, 1870, 20, neuer folge 7 : 223.

solution the field of vision is obscured, the dividing line of the two halves, or the double line in the triple field, becomes blurred or invisible and the intensity of illumination is diminished. A colored liquid which is bright is far more easy to polarize than a colorless liquid which is turbid. In fact, it is only rarely in sugar work that samples will be found which require any special decolorizing treatment other than that which is received in applying the reagents which serve to make the solutions limpid. In the following paragraphs the approved methods of clarifying sugar solutions preparatory to observation in the polariscope will be described.

107. Alumina Cream.—The hydrate of alumina, commonly known as alumina cream, is always to be preferred as a clarifying agent in all cases where it can be successfully applied.* It is a substance that acts wholly in a mechanical way and therefore leaves the sugars in solution unchanged, carrying out only suspended matters. In the preparation of this reagent a solution of alum is treated with ammonia in slight excess, the aluminum hydroxid produced washed on a filter or by decantation until neutral in reaction. The hydroxid is suspended in pure water in proportions to produce a creamy liquid. Although apparently very bulky, the actual space occupied by the amount of hydroxid is so small as to produce no disturbing effect of importance on the volume of the sugar solution. The cream thus prepared is shaken just before using and from one to five cubic centimeters of it, according to the degree of turbidity of the saccharine solution, are added before the volume in the flask is completed to the mark. After filling the flask to the mark the ball of the thumb is placed over the mouth and the contents well shaken and allowed to stand for a few moments before filtering. The method of preparing the alumina cream prescribed by the Association of Official Agricultural Chemists is as follows:—A concentrated potassium or ammonium alum solution is divided into original portions. To the larger portion add a slight excess of ammonium hydroxid and to this mixture add slowly the

* Spencer, Handbook for Cane-sugar Manufacturers, 4th Edition, 1906

smaller portion of the alum solution until a fairly acid reaction is secured.

The alumina cream is well suited to use with solutions of commercial sugars of not too low a grade and of most honeys and high grade sirups. It is usually not powerful enough to clarify beet and cane juices, molasses and massecuites but may be used with other reagents to effect a quicker subsidence of the precipitate and to facilitate filtering. Finely divided kaolin that has been thoroughly dried and added after making up to volume removes cloudiness in some substances much better than alumina cream.

108. Basic Lead Acetate.—A solution of basic lead acetate is an invaluable aid to the sugar analyst in the preparation of samples for polarimetric observation. It acts as a clarifying agent by throwing out of solution certain organic compounds and by uniting with the organic acids in solution forms an additional quantity of precipitate, and these precipitates act also mechanically in removing suspended matters from solution. The action of this reagent is therefore much more vigorous than that of alumina cream. Coloring-matters are often precipitated and removed by treatment with basic or neutral lead acetate. It happens therefore that there are few samples of saccharine bodies whose solutions cannot be sufficiently clarified by lead acetate to permit of polarimetric observation.

The reagent is most frequently employed of the following strength:⁹ Boil for half an hour in one and a half liters of water 464 grams of lead acetate and 264 grams of litharge with frequent stirring. When cool, dilute with water to two liters, allow to stand until clear, and decant the solution. The specific gravity of this solution is about 1.267.

In a solution of basic lead acetate of unknown strength the percentage of lead acetate may be determined from its specific gravity by the following table:¹⁰

⁹ Tucker's *Manual of Sugar Analysis*: 164.

¹⁰ Spencer's *Handbook for Sugar Manufacturers*: 91.

PERCENTAGE OF LEAD ACETATE CORRESPONDING TO DIFFERENT
SPECIFIC GRAVITIES AT 15°.

Specific gravity	Percentage of lead acetate	Specific gravity	Percentage of lead acetate
1.0127	2	1.2040	28
1.0255	4	1.2211	30
1.0386	6	1.2395	32
1.0520	8	1.2579	34
1.0654	10	1.2768	36
1.0796	12	1.2966	38
1.0939	14	1.3163	40
1.1084	16	1.3376	42
1.1234	18	1.3588	44
1.1384	20	1.3810	46
1.1544	22	1.4041	48
1.1704	24	1.4271	50
1.1869	26		

According to Browne there are two or three well marked lead subacetates.¹¹

These have the following composition:— $3\text{Pb}(\text{Ac}).2\text{PbO}$; $\text{Pb}(\text{Ac}).1\text{PbO}$ and $\text{Pb}(\text{Ac}).2\text{PbO}$.

109. Manipulation.—This solution is added to the flask containing the weighed portion of the material to be tested before the volume is completed. On addition of a small quantity from a pipette a precipitate forms and on the gradual addition of small quantities a point is reached where no further precipitation occurs. An excess of the clarifier is to be avoided. In general, from $\frac{1}{2}$ to 10 cubic centimeters of the solution is necessary.

110. Errors Due to Use of Lead Solutions.—In the use of lead solutions there is danger of errors in the results of the work. These errors are due to various sources. Lead subacetate solution, when used with low grade products, or sugar juices, or sirups from beets and canes, precipitates albuminous matters and also the organic acids present. The bulk occupied by these combined precipitates is often of considerable magnitude, so that on completing the volume in the flask the actual sugar solution present is less than indicated. The resulting condensation tends to give too high a polarimetric reading. With pure samples this error is of no consequence, but especially with low grade sirups

¹¹ Bureau of Chemistry, Bulletin 122, 1910 : 223.

and molasses it is a disturbing factor, which must be considered.

111. Double Dilution.—One of the best methods of correcting it has been proposed by Scheibler.¹² To 100 cubic centimeters of a solution of the sample, ten of lead solution are added, and after shaking and filtering the polarimetric reading is taken. Another quantity of 100 cubic centimeters, of the solution with ten of lead is diluted to 220 cubic centimeters, shaken, filtered and polarized. Double the second reading, subtract it from the first, multiply the difference by 2.2, and deduct the product from the first reading. The remainder is the correct polarization.

The process just described is adapted to the usual work with beet juices and sirups. For cane juices measured by the graduated pipette, hereafter to be described, and for weighed samples of molasses and massecuites, the following method of calculation is used.¹³ To the sample dissolved in water, add a measured portion of the lead subacetate solution, make its volume 100 cubic centimeters and observe the polarimetric reading. Prepare a second solution in the same way and make the volume double that of the first and again take the polarimetric reading. Multiply the second reading by two, subtract the product from the first reading and multiply the remainder by two, and subtract the product from the first reading.

Example.—First polarization30.0
 Second polarization14.9
 Then $30 - (2 \times 14.9 = 29.8) = 0.2$
 $0.2 \times 2 \dots\dots\dots = 0.4$
 and $30 - 0.4 \dots\dots\dots = 29.6$

The corrected reading therefore shows that the sample contained 29.6 per cent. of sugar.

¹² Zeitschrift des Vereins für die Rubenzucker-Industrie, 1875, **25**; neuer folge **12** : 1054.

Spencer, Handbook for Cane-Sugar Manufacturers, 1906 : 84.

Wiechmann, International Sugar Journal, 1903, **5** : 379; 1905, **7** : 395, 1906, **8** : 11.

¹³ Deer, International Sugar Journal, 1907, **9** : 122.

112. Error Due to Action of Lead Subacetate on Levulose.—In the use of lead subacetate solution not only is there danger of error due to the causes just described, but also to a more serious one, arising from the chemical interaction of the clarifying agent and levulose.

Lead subacetate forms a chemical union with levulose and the resulting compound has a different rotatory power from the left-handed sugar in an uncombined state. Again this compound is often carried down with the heavy precipitate and hence lost to the solution. By adding a sufficient quantity of subacetate solution, the left-handed rotation of levulose may be greatly diminished if not entirely destroyed. In this case the dextrose, which with levulose forms inverted sugar, serves to increase the apparent right rotation. The reading of the scale is therefore higher than would be given by the sucrose alone. If the lead subacetate could be added in just the proportion to make the invert sugar neutral to polarized light, its use would render the analysis more accurate; but such a case could only arise accidentally. To correct the error in part, after clarification, the compound of levulose and lead may be decomposed by the addition of acetic acid according to the method of Spencer converting the basic into the normal salt. In this case the true content of sucrose can only be obtained by the method of inversion proposed by Clerget, which will be described in another paragraph.

It is evident that the errors due to the clarification of a sugar solution containing invert and reducing sugars are to a certain extent cumulative if sucrose alone is to be determined. The volume of the lead precipitate tends to increase the rotation by diminishing the volume of the solution and the action of the lead salt on the levulose tends to increase the rotation by diminishing its left-handed solution. Bryan has subjected the various changes to an experimental study which may be consulted for further details.¹⁴

113. Horne's Dry Lead or Dry Basic Lead Acetate.—In order to eliminate as much as possible the error due to the volume of the

¹⁴ Bureau of Chemistry, Bulletin 116, 1908 : 71.

precipitate, Horne¹⁵ proposed the use of a dry basic lead acetate to be added to the solution requiring clarification after the volume had been made up. This dry lead has the composition of the basic lead usually used, viz., $3\text{Pb}(\text{Ac})$, 2PbO ., and contains 72.77 per cent. of PbO . Browne,¹⁶ however, found that preparations of this reagent on the market varied considerably in composition, although that manufactured according to Horne was of very even composition. The preparation in powdered form is added to the solution after it is made up to volume. The precipitate formed does not affect the volume. Its use, however, in excess tends to increase the volume of the solution by the quantity remaining undissolved. Horne¹⁷ states that the addition of one gram causes an increase in volume of 0.22 cubic centimeter, and Bryan¹⁸ finds it to be 0.16 cubic centimeter. It is seen that care should be exercised in its use to prevent an excess. Dry lead subacetate affects reducing sugar in the same way as the solution.

The use of dry subacetate of lead as a clarifier for polarization of solutions containing reducing sugar is to be recommended over the use of the solution of this salt when sucrose only is to be determined. With subacetate solution the errors produced by the volume of precipitate and by its action on reducing sugars are on the same side, while with the dry subacetate the errors of these two are opposite and often very nearly compensatory. Like the solution of basic lead acetate the dry salt should not be used in clarifying solutions for reducing sugar determinations. Objections have been raised to the use of dry subacetate by Pellet¹⁹ and some other chemists have noted that quite a large quantity of it is necessary to clarify molasses, as the particles of lead subacetate become coated with the precipitate and the remaining substance is inactive. Horne, however, recommends

¹⁵ Journal of American Chemical Society, 1904, **26** : 191.

¹⁶ Bureau of Chemistry, Bulletin 122, 1910 : 273.

¹⁷ Journal of American Chemical Society, 1907, **79** : 928.

¹⁸ Bureau of Chemistry, Bulletin 122, 1910 : 177.

¹⁹ Bulletin de l'Association des chimistes de Sucrerie et Distillerie, 1905-6, **23** : 285-97.

the addition of a little fine dry sand and at the same time as the dry lead salt is added. In shaking this mixture, these aggregates are easily broken up and the lead salt quickly dissolved.

114. Basic Lead Nitrate or Herles Solution.—This reagent is used extensively in beet sugar work by many Austrian and German sugar chemists. Its use has been tried by the Association of Official Chemists and found to give results equal to basic lead acetate. The clarification produced by it in many cases is better than by the acetate. The reagent was originally prepared by dissolving 100 grams of sodium hydroxid in two liters of water and 1,000 grams of lead nitrate in the same volume. The two solutions were mixed, the resulting precipitate collected, washed and finally suspended in water to be used in that form. The reagent as recommended by the Austrian sugar chemists is prepared in the same way, but instead of pouring the two solutions together and using the precipitate for clarifying, they make the solutions independently and then use equal portions of each for clarification. The reagent is added to the sugar solution before the volume is completed. Its use is preferred because of the greater brightness of the solution. Both dry lead subacetate and Herles solution were recommended for constant use by the Association of Official Agricultural Chemists at the annual meeting in November, 1911.

115. Error Due to Change of Specific Rotation of Sugars by Lead.—Besides the two errors mentioned, there is another which deserves attention, viz., the effect of lead salts on the specific rotation of the sugars. The rotary power of sucrose is not markedly modified by subacetate of lead under the usual analytical conditions. Bates and Blake²⁰ found that up to six cubic centimeters added to pure sucrose solution, there was a slight decrease in rotation of sucrose and beyond that quantity the rotation increased in direct proportion to the quantity of the reagent added. They conclude that its influence is of special significance in the polarimetric determinations of sucrose in

²⁰ Journal of the American Chemical Society, 1907, 29 : 286.

raw sugars, and is of sufficient magnitude to place it along with the error introduced by the volume of the precipitate, the temperature coefficient, and the presence of invert sugar and other impurities.

In alcoholic solutions of sucrose there is a decided diminution of rotation produced by lead salts. Acetates and alkalis have little effect on the rotation of sucrose, although there is a slight diminution of the rotation, especially in concentrated solutions.

With d-glucose neither basic lead acetate nor neutral lead acetate exerts any influence upon the rotatory power of aqueous solutions under the ordinary analytical conditions.²¹ Alkalis and acetates have no effect on the rotation of dextrose in the concentration generally employed.

With fructose (levulose) the rotation is very greatly diminished by the presence of basic lead acetate. Acetic acid added to acidity restores the rotation of the fructose.²²

116. Agents for Removal of Lead from Solution.—When lead salts have been added in excess or are removed before inversion of the solution with acid, various substances are proposed for this purpose. Among these are solutions of sodium chlorid, sodium carbonate, di-sodium phosphate, sodium sulfate and sodium, potassium or ammonium oxalate. In using these solutions a measured portion of the filtered liquid is treated with one of these substances, and the mixture made up to a definite volume. In order to avoid this operation some of these salts in dry form have been proposed, and especially dry sodium carbonate. Dry potassium oxalate produces a complete precipitation of the lead without darkening the color of the solution, but it contains one molecule of water of constitution, hence if used in quantity, would introduce an error. Lately, sodium oxalate has been recommended. It is less soluble than the potassium salt, but can be obtained without the molecule of water. Sodium sulfate has not proved to be satisfactory, in that it does not com-

²¹ Pellet, Bulletin de l'Association des Chimistes de Sucrerie et de Distillerie 14 : 28, 141.

²² Gill, Journal of American Chemical Society, 1871, 24 : 91.

pletely remove the lead. When clarifying a solution of sugar, the precipitate caused by the clarifier should be separated before the substance used for removing lead salts is added. In other words, the latter should be a separate operation.

117. Summary.—The following general summary is given of the principal conclusions based on the study of the action of lead salts as clarifiers.

(1) Basic lead acetate when used in solution or in the dry condition causes a precipitation of levulose and dextrose in the presence of substances precipitable by lead, and hence should not be used as a clarifying agent when reducing sugars are to be determined.

(2) In the clarification of solutions for the determination of reducing sugars, neutral lead acetate should be used or any other defacting agent which does not invert sucrose nor precipitate the reducing sugars present.

(3) In the preparation of solutions of lead subacetate for clarifying sugar solutions for polarization the content of basic lead should be controlled as well as the specific gravity.

(4) In the clarification of raw cane sugars for polarimetric examinations, the use of anhydrous lead subacetate, Horne's method, gives more accurate results than any other form of lead clarification.

118. Clarification with Mercuric Compounds.—Where the disturbing bodies in a solution are chiefly of an albuminoid nature, one of the best methods of securing clarification is by the use of a solution containing an acid mercuric compound.²³ In the case of milk this method is to be preferred to all others. Albuminoid bodies themselves, have the property of deflecting the plane of polarization, as a rule, to the left, and therefore, should be completely removed from solutions containing right-handed sugars such as lactose. For this purpose the mercuric compound is more efficient than any other. It is prepared and used as follows: Dissolve mercury in double its weight of strong nitric acid and dilute the solution with an equal volume

²³ Wiley, American Chemical Journal, 1884, 6 : 289, 301.

of water. One cubic centimeter of this solution is sufficient to clarify fifty times its volume of milk.

The solution is also prepared by the following method.²⁴

To 160 cubic centimeters of HNO_3 (specific gravity 1.39) is added gradually 220 grams of red mercuric oxid, well shaken and 160 cubic centimeters of water added. The mixture is slowly heated to the boiling-point until all the oxid is dissolved. The solution is cooled and almost neutralized with 63 cubic centimeters of a five per cent. sodium hydroxid solution, diluted to one liter filtered and preserved in dark bottles.

Patein and Dufau prepare the reagent as follows:²⁵ To 200 grams of yellow mercuric oxid and 400 cubic centimeters of water in an evaporation dish, add cautiously sufficient nitric acid (about 140 cubic centimeters) to just dissolve the oxid and sodium hydroxid until a permanent precipitate is just formed. Dilute the whole to one liter and filter. As this solution tends to become more acid with age, by the deposition of basic mercury salts, it should receive a little alkali from time to time.

The reagent as prepared by either of the above methods is added with constant shaking to the sugar solution from a graduated pipette and when a sufficient quantity has been added a solution of half normal sodium hydroxid is added while shaking until the solution is neutral, otherwise the sucrose will become inverted. The flask is filled to the mark with water and polarization made after filtration. A correction for ordinary sugar work equivalent to 0.2 cubic centimeter for each 10 cubic centimeters of the reagent for its volume is to be made.²⁶

119. Zinc Dust.—This is never used as a clarifier of solutions for the direct polarization, but has been recommended to decolorize the solutions after acid inversion. For this purpose not over one gram of pure zinc dust is added to the acid solution and, when all dissolved, the polarization is made. With one

²⁴ International Sugar Journal, 1909, 11 : 607.

²⁵ Zeitschrift für Untersuchung der Nahrungs und Genussmittel, 1902, 5 : 726.

²⁶ International Sugar Journal, 1909, 11 : 607.

gram zinc dust no effect on the rotation of the invert sugar was noted by Davoll.²⁷

120. Decolorization by Means of Bone-Black.—Where the means already described fail to make a solution sufficiently colorless to permit of the passage of a ray of polarized light, recourse should be had to other agents. The most efficient of these is bone-black. For laboratory work it is finely ground and should be dry if added to an already measured solution. When moist it should be added to the flask before the volume is completed, and a correction made for the volume of the dry char employed. Bone-black has the power of absorbing a certain quantity of sugar, and for this reason as little of it should be employed as is sufficient to secure the end in view. If not more than one gram of the char be used for 100 cubic centimeters of solution, the error is not important commercially. The error may be avoided by placing the char on the filter and rejecting the first half of the filtered solution. The char becomes saturated with the first portion of the solution, and does not absorb any sugar from the second. This method, however, does not secure so complete a decolorization as is effected by adding the black directly to the solution and allowing to stand for some time with frequent shaking.

121. Hydrosulfites.—The sodium, calcium, aluminium or double salts of a hydrosulfurous acid $\text{H}_2\text{S}_2\text{O}_4$ have been recommended for decolorizing sugar solutions.²⁸ These salts are easily broken up into acid sulfites and hydrogen, both being bleaching agents.²⁹ They are offered in powdered form under various names such as "Blankit," "Redo," "Hydrosulfite B. A. S. F." etc. These reagents must be kept in air-tight packages since the moisture in the air decomposes most of them. In powdered form they are added to the sugar solution after the volume is made. A very small quantity is sufficient. They may be added to the solution after clarifying with neutral lead

²⁷ Journal of American Chemical Society, 1903, **25** : 1027.

²⁸ Zeitschrift für angewandte chemie, 1904, **17** : 1448.

Berichte der Deutschen Chemischen Gesellschaft, 1906, **39** : 2811.

²⁹ Centralblatt für des Zucker-Industrie, 1907, **15** : 975.

and removal of the dissolved lead. Some trouble is experienced with their use from the precipitation of sulfur. This can be easily removed by filtering the solution through kaolin. In their use there is no error due to the volume of the precipitate but they affect the rotation of dextrose and in some cases, especially where used in excess, invert some of the sucrose.⁸⁰

Instead of adding the hydrosulfite to the sugar solution in the flask, a small crystal may be added to the polarization tube and this shaken and polarized immediately.

122. Hypochlorites.—The bleaching action of chlorid of lime was tried by Zameron⁸¹ on sugar products and found to give good solutions for polarizing without influence on sugars.

123. Remarks on Analytical Process.—Since large weights of sugar are taken for polarization, a balance which will weigh accurately to one milligram may be used. In commercial work the weighing is made in a counterpoised dish with a prominent lip, by means of which the sample can be directed into the mouth of the flask after partial solution. Where the air in the working room is still, an uncovered balance is most convenient. With a little practice the analyst will be able to dissolve and transfer the sample from the dish to the flask without danger of loss. The source of light used in polarizing should be in another room, and admitted by a circular opening in the partition. In a closed polarizing room, which results from the darkening of the windows, the temperature will rapidly rise if a lamp be present, endangering notably the accuracy of the work, and also interfering with the comfort of the observer. The greatest neatness must be practiced in all stages of the work, and especially the trough of the polariscope must be kept from injury which may arise from the leaking of the observation tubes.

124. Constant Temperature Rooms.—In order to eliminate errors due to changes in temperature, rooms with thermostatic control are desirable. The principle on which the construction

⁸⁰ Bureau of Chemistry, Bulletin 116, 1908 : 71, 76.

⁸¹ Bulletin de l'Association des Chimistes de Sucrerie et de Distillerie, 1895, 16 : 337.

of such rooms is based rests upon the proper cooling or warming devices which automatically produce and preserve any desired temperature. This is done by leading into the closed insulated chamber cool or warm currents of air. The air is cooled by passing over cold brine coils or warmed with steam coils as the case may be. Fig. 49 shows the outside view of such a room with the varied apparatus required to automatically control the temperature and Fig. 50 the inside of the room show-

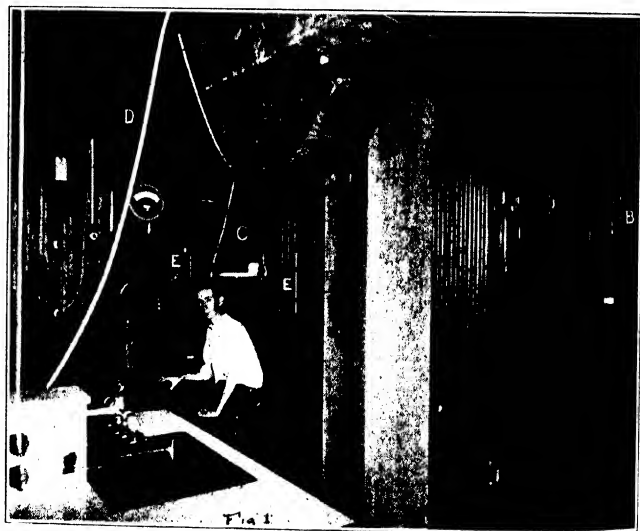


Fig. 49.—Outside of Constant Temperature Room.

ing the arrangements of the polariscope. As few lights are kept burning as is possible and the lamps that illuminate are on the outside of the observation room.

In both cases the temperature is controlled by a system of automatic dampers, thermostats being installed in the polarization rooms to operate these dampers. As there is only one air supply both thermostats act on the same dampers but to make the room independent a switch is provided so that either thermostat provided can control the temperature.

The research room is equipped for three polariscopes, the other for four. The instruments stand on slabs of alberine stone supported on wooden frames built in the rooms. The lighting of these rooms is secured by four systems. First, lamps suspended from the ceiling for general light, controlled by a switch near the door. Second, lamps for polarization carried on supports outside of the room and controlled by a switch on the table frame in front of and below each instrument, as shown in the

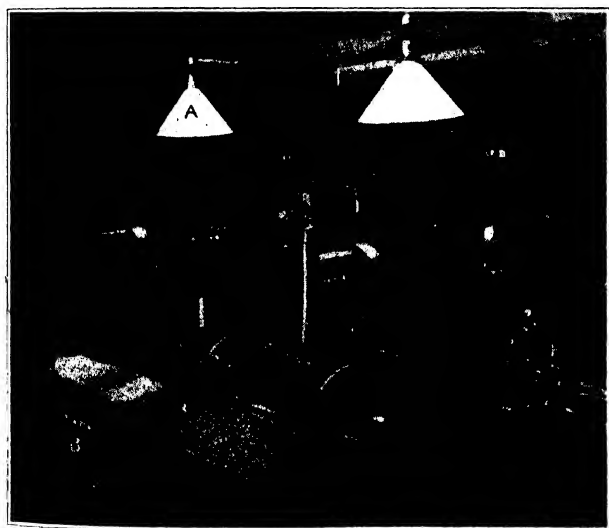


Fig. 50.—Constant Temperature Room.

figure (Fig. 49). These lights are stereopticon lamps having coil filaments and give locally a very intense glow. Each lamp is independent. Third, independent lamps in green shades over each instrument, as shown in Fig. 50, for use when changing tubes, etc., having a switch beside the one mentioned above. Fourth, a six volt circuit from the storage battery for illuminating the scale of the instrument in a manner not to dazzle the eye. These small lamps are operated independently by push buttons

placed beside the other two switches. The openings through the wall for illuminating the polariscopes are an inch in diameter and are covered on each side by ground glass plates.

In making polarizations in this room the material is weighed and transferred to the flask at ordinary room temperature as near 20° as possible. The flasks are then brought into the polarization room, and clarified with the necessary clarifying agents, also stored here, filled to the mark with water that has been stored in this room and consequently at 20° . The polarization tubes are kept in this room, the filtration is performed, the tubes filled and readings made therein. In addition to the apparatus for polarization, an electric light has been placed low on the wall above the table to be used for making refractometer readings. By keeping a sample over night in the room, the refractometer reading can be made at 20° without the use of the cooling chamber in the late form of zeiss refractometer.

A hot water apparatus has been installed outside of the laboratory consisting of a large copper tank shown in Fig. 49 heated from below with an Argand burner with a tube and valve entering the room. To this is attached a glass tube at right angles to give the height of water in the outside tank. The valve consists of two portions, one of which is fitted with a large stop-cock which can be opened and a fast flow of hot water obtained or by closing this and opening a needle valve a small supply of water can be admitted. This valve and connection is shown in Fig. 50. To the end of this is attached a rubber tube which connects with the jacketed tube placed in the polariscope and from the other end of the jacketed tube runs another rubber tube to the waste pipe. By these means polarizations can be made quickly at 87° or any temperature desired.

125. Determination of Sucrose by Inversion.—In the foregoing paragraphs directions have been given for the estimation of sugar (sucrose) by its optical properties. It has been assumed so far, that no other disturbing bodies have been present, save those which could be removed by the clarifying agents described. The case is different when two or more sugars are present, each of which has a specific relation to polarized light. In such cases

some method must be used for the optical determination of sucrose, which is independent of the influence of the other polarizing bodies, or else recourse must be had to other methods of analysis. The conversion of the sucrose present into invert sugar by the action of an acid or an enzyme, affords an opportunity for the estimation of sucrose in mixed sugars, by purely optical methods. This process rests upon the principle that by the action of a dilute acid, or of an enzyme, the sucrose is completely changed, while other sugars present are not sensibly affected. Neither of these assumptions is rigidly correct but each is practically applicable.

The sucrose by this process of hydrolysis is converted into an equal mixture of levulose and dextrose. The former, at room temperatures, has the higher specific rotating power, and the deflection of the plane of polarization in a solution of inverted sugar is therefore to the left. The levo-rotatory power of invert sugar varies with the temperature, and this arises from the optical properties of the levulose. The influence of temperature on the rotating power of sugars is perceptible in every case, and in the case of sucrose is of sufficient importance to require special methods of correction.

This method of analysis is invaluable in control work in factories, in the customs' and in agricultural laboratories. Since the rotating power of levulose diminishes as the temperature rises, an accurate thermometric observation must accompany each polarimetric reading. At about 88° the rotatory powers of dextrose and levulose are equal, and a solution of pure invert sugar examined at that degree, is found to be neutral to polarized light.

126. Clerget's Method of Inversion.—The classical method of Clerget for the determination of cane-sugar by double polarization before and after inversion is now practiced universally in sugar laboratories.³²

Clerget points out first the observation of Mitscherlich regarding the influence of temperature on the rotatory power of invert

³² Memoir presented to *La Société d'Encouragement des Industries Nationales*, November, 1846.

sugar, and calls attention to the detailed experiments he has made which resulted in the determination of the laws of the variation. From these studies he was able to construct a table of corrections, applicable in the analysis of all saccharine substances in which the cane-sugar is polarized before and after inversion. The basis of the law rests upon the observation that a solution of pure sugar, polarizing 100° on the sugar scale, before inversion, will polarize 44° to the left at a temperature of zero after inversion. The quantity of sugar operated upon by Clerget amounted to 16.471 grams in 100 cubic centimeters of solution. On the instrument employed by him this quantity of sugar in 100 cubic centimeters gave a reading of 100° to the right on the sugar scale when contained in a tube twenty centimeters in length. The process of inversion carried only by Clerget is as follows:

The sugar solution is placed in a flask, marked on the neck at 100 and 110 cubic centimeters; or if half quantities are used, in a flask marked on the neck at 50 and 55 cubic centimeters. The flask is filled with the sugar solution to the first mark and then a sufficient quantity of strong hydrochloric acid added to bring the volume of the liquid to the second mark. The mouth of the flask is then closed with the thumb and its contents thoroughly mixed by shaking. A thermometer is placed in the flask which is set in a water-bath in such a way that the water comes just above the level of the liquid in the neck of the flask. The water is heated in such a manner as to bring the temperature of the contents of the flask, as determined by the thermometer, exactly to 68° and at such a rate as to require fifteen minutes to reach this result. At the end of fifteen minutes the temperature having reached 68° the flask is removed and placed at once in another water-bath at the temperature of the room, to which temperature the contents of the flask are cooled as rapidly as possible. To make the polarimetric observation, a tube 22 centimeters in length is filled with the inverted sugar solution by means of a tubulure in its center, which serves not only the purpose of filling the tube but also afterwards to carry the thermometer, by means of

which the temperature of observation can be taken. If the sugar solution be turbid, or contain any lead chlorid due to the previous use of basic lead acetate in clarification, it should be filtered before being introduced into the observation tube. This tube being one-tenth longer than the original compensates for the dilution caused by the addition of the hydrochloric acid in inversion.

When reading, the bulb of the thermometer should be withdrawn far enough to permit the free passage of the ray of light and the exact temperature of the solution noted.

The above outline of Clerget's method of inversion is given in order that the analyst may compare it with any of the variations which he may find in other works. The chief points to which attention is called, are, first, the fact that only a little over sixteen grams of sugar are used for ten cubic centimeters of strong hydrochloric acid, and second, that the time of heating is exactly fifteen minutes, during which time the contents of the flask should be raised from room temperature to exactly 68°.

From the above it is seen that the process of Clerget, as originally described, can be applied directly to all instruments, using approximately sixteen grams of sugar in 100 cubic centimeters. Experience has also shown that even when larger quantities of sugar are employed, as for instance, approximately twenty-six grams, the inversion is effected with practical completeness in the same circumstances.

127. Modified Methods.—At the present day, three methods of conducting the inversion are approved, viz., the Herzfeld modification of the German official method, the Tolman method or inversion in the cold, and the use of invertase.

128. Herzfeld Method.³³—Half normal weight of the material (13 or 13.024 grams) is dissolved in 75 cubic centimeters of water in a 100 cubic centimeter flask, and five cubic centimeters of hydrochloric acid added (1.13 specific gravity). Place the flask in a water-bath at 70° which will bring the temperature of the solution to 69° in two and one-half to three minutes. Maintain a temperature of as nearly 69° as possible from seven to

³³ Zeitschrift des Vereins für die Rübenzucker-Industrie, 1888, 38 neuer folge, 25 : 699-702.

seven and one-half minutes, making a total time of heating of ten minutes. During this time, the temperature must not go over 70° or below 67° . Remove the flask and cool immediately to 20° and dilute to 100 cubic centimeters. Polarize the solution in a 200 cubic centimeter tube. Under these conditions, the inverted solution of pure sucrose will polarize -42.66 at 0° . The Association of Official Agricultural Chemists have adopted this method, but with a slight modification, viz., instead of using a separate portion of the material for inversion, a portion of the solution in the direct polarization is used. The lead is removed from the solution before inversion. The method is then followed as above described.

129. Tolman's Method or Inversion in the Cold.⁸⁴—To avoid change in color due to heating, Tolman tried the effectiveness of inversion of sucrose at ordinary room temperatures. The advantages of such a method are, first, that fructose would not be so liable to destruction, as by the heating method, and, second, where many determinations are to be made, the solutions could be set away and polarized later, allowing other work to go on at the same time. Hammerschmidt⁸⁵ worked out the time necessary for the complete inversion of 13.024 grams of sucrose at different temperatures and with varying amounts of hydrochloric acid. His results for the ordinary room temperatures are given in the following table:

Temperature, $^{\circ}\text{C}$.	—Hydrochloric acid, sp. gr. 1.18—	
	5 cc.	10 cc.
10	225.6 hrs.	94.4 hrs.
15	101.7 hrs.	44.5 hrs.
20	47.5 hrs.	20.9 hrs.
25	23.3 hrs.	10.2 hrs.
30	10.6 hrs.	3.1 hrs.

Tolman found that at from 20° to 24° complete inversion of 13.024 grams takes place in from 10 to 20 hours, while at 25° only 10 hours are required. He proposed a method which is considered official by the Association of Official Agricultural

⁸⁴ Bureau of Chemistry, Bulletin 73, 1903 : 72.

⁸⁵ Zeitschrift des Vereins für die Rübenzucker-Industrie, 1890, 49, neuer folge, 27 : 473.

Chemists. The procedure is the same as for Herzfeld's method up to the point of inversion. After the acid is added, the flask is set aside for at least 20 hours, and then made to volume and polarized.

Comparison of this method with the official German method, carried on under correct conditions, gave agreeing results. A sugar solution polarizing 100° before inversion by the German method gave -32.86 at 20° after inversion, and by the cold inversion -32.88 .

130. Influence of Strength of Solution, Amounts of Acid and Time of Inversion on the Results.—As has been intimated, the strength of a sugar solution at the time of heating together with the quantity of hydrochloric acid used are factors that must be considered.

Considering the amount of acid used Hammerschmidt³⁶ gives a table showing the rotation of sugar solutions with varying amounts of hydrochloric acid:

Weight of sucrose grams.	Polarization $^\circ$ V with hydrochloric acid—			
	5 cc.	10 cc.	15 cc.	20 cc.
26.048	34.00	35.04	35.95	36.80
13.024	33.00	34.12	35.15	36.03

The results with 13.024 grams of sucrose have been doubled, so as to compare with the 26.048 grams. Tolman found also an increase in the various polarization of invert sugar when strong hydrochloric acid was added. Browne³⁷ found that by neutralizing an inverted solution, the rotation was diminished, viz., -32.5 in the acid solution, and -31.7 after neutralization. It is seen that hydrochloric acid does increase the levorotation of invert sugar or diminish that of levulose.

As regards time of heating in inversion, Bornträger and Herzfeld³⁸ shows that the resulting rotation is decreased by increasing the length of time of heating.

³⁶ Zeitschrift des Vereins für die Rübenzucker-Industrie, 1890, **40**, neuer Folge, **27**: 477.

³⁷ Bureau of Chemistry, Bulletin 110, 1908: 44.

³⁸ Zeitschrift des Vereins für die Rübenzucker-Industrie, 1888, **38**, neuer Folge, **25**: 707.

Zeitschrift des Vereins für die Rübenzucker-Industrie, 1890, **40**, neuer Folge, **27**: 893.

131. Influence of Strength of Solution and Time of Heating on the Inversion of Sucrose.—The Clerget formula holds good only for the conditions specified and these conditions must be rigidly adhered to in order to secure the proper results. This matter has been thoroughly studied by Bornträger, who also gives a nearly complete bibliography of the subject. As a result of his investigations it seems well established that the original Clerget formula is practically correct for the conditions indicated. Bornträger modifies it only by substituting in the formula 143.66 for 144. This is so nearly the same as the Clerget factor that it is not advisable to substitute it therefor. If, however, the inverted sugar solution be diluted to double its volume before polarization the factor proposed by Landolt, viz., 142.4 gives more nearly accurate results. So many factors enter into the determination of the value of K (the sum of the direct and invert polarizations at 0°) that it is impractical to fix it at a definite figure. If the hydrochloric acid be neutralized before polarization by an alkaline body, the character of the salt which is formed also influences, to a greater or less extent, the specific rotatory power of the solution. Hydrochloric acid itself also influences the rotation to a certain degree.

132. Calculation of Results.—The percentage of sucrose in a solution which has been polarized before and after inversion is calculated by an appropriate formula from the data obtained or is taken directly from tables. These tables are too long to insert here, and in point of fact the calculation can be made from the formula almost as quickly as the result can be taken from a table.

The formula for calculating the percentage of sucrose by Clerget is as follows:

$$\text{Sucrose} = \frac{\text{Direct polarization} - \text{Invert polarization}}{K \times \frac{t}{s}}$$

In which, K = constant, and t = temperature at which polarizations have been made. When one polarization is + and the other — the two values are added, when they are both + or both — the invert polarization is subtracted from the direct. In the

Herzfeld method, and also in Tolman's method, when pure sugar is the substance polarized, the value of K is 142.66. Herzfeld³⁹ has published a list of values of K for varying quantities of sucrose in the solution taken for inversion which appear in the following table.

Grains of sucrose in the 100 cc.	Constant K	Grains of sucrose in the 100 cc.	Constant K
1	141.85	11	142.52
2	141.91	12	142.59
3	141.98	13	142.66
4	142.05	14	142.73
5	142.12	15	142.79
6	142.18	16	142.86
7	142.25	17	142.93
8	142.32	18	143.00
9	142.39	19	143.07
10	142.46	20	143.13

For more accurate work, these values should be substituted in the equations for 142.66, after a preliminary calculation has been made to show relatively how much sucrose is present. Herzfeld has also made a change in the Clerget formula to take into account the varying constant.

$$\text{Sucrose} = \frac{\text{Direct polarization} - \text{Invert polarization}}{141.84 \pm \frac{t}{20} - \frac{t}{2}}$$

In this i = the invert polarization obtained. Tolman⁴⁰ gives the following formula to correct for the change of rotation of invert sugar due to hydrochloric acid added for inversion as he believes that this is the cause of the variation and not the concentration of the sucrose.⁴¹

$$\text{Sucrose} = \frac{a - [b - (0.062 \times b)]}{141.79 - \frac{t}{2}}$$

In which a = direct polarization.

b = invert polarization calculated to normal weight.

t = temperature of observation.

³⁹ Zeitschrift des Vereins für die Rübenzucker-Industrie, 1890, **40**, neuer folge, **27**: 194.

⁴⁰ Bureau of Chemistry, Bulletin 73, 1903: 75.

⁴¹ Journal of the American Chemical Society, 1902, **24**: 523.

133. Precautions to be Observed.—In inversion work the following points are to be borne in mind, viz., the removal of all lead before adding acids, the neutralization of any alkali present before adding the acid and securing the same temperature of reading before as after inversion, and as near 20° as possible. Even with these precautions the results are open to criticism unless the solutions operated on are nearly pure sugars.

134. Inversion by Means of Invertase.—Instead of using acids for the inversion of cane-sugar the hydrolysis can be easily effected by means of an enzyme derived from yeast. A complete history of the literature and characteristics of this ferment, together with a study of its properties and the various methods of preparing it, has been given by O'Sullivan and Tompson.⁴² In the preparation of invertase, they found the following method most effective:

The yeast is allowed to liquefy for at least a month in a fairly warm room without stirring. At the end of this time the surface is removed and any supernatant liquid poured away. The lower sedimentary part is thrown on a quick-acting filter and allowed to drain for two days. To the filtrate, alcohol of specific gravity 0.87 is gradually added to the extent of one and a half times its volume with continued and vigorous stirring. The process of adding the alcohol and stirring should require about half an hour, after which the mixture is allowed to stand for twenty-four hours to allow the precipitated invertase to settle. The supernatant liquid is poured away and the precipitate washed several times on successive days by decantation with alcohol of 0.92 specific gravity. When the washings become nearly colorless the precipitate is thrown on a filter, allowed to drain, and immediately removed and mixed with a large bulk of alcohol of 0.92 specific gravity. The precipitate is again collected, mixed thoroughly with its own bulk of water, and some alcohol of 0.97 specific gravity, allowed to stand for a few hours and thrown on a filter. The filtrate contains the invertase. Yeast may also be used directly as the inverting agent.

⁴² Journal of the Chemical Society Transactions, 57 : 834.

135. Determination of Activity of Invertase.—The activity of a solution of invertase, prepared as above, is measured by the number of minutes required for it to reduce to zero the optical power of a solution of 100 times its weight of cane-sugar at a temperature of 15°.5. In order to facilitate the action of the invertase, a trace of sulfuric acid is added to the solution. The manipulation is as follows:

Fifty grams of sucrose are dissolved in water and made to a volume of nearly a quarter of a liter and placed in a bath maintained at 15°.5. Half a gram of the invertase is added, the time noted, the solution immediately made up to a quarter of a liter and well shaken. The contents of the flask are poured rapidly into five beakers; the actual quantity in each beaker is not necessarily the same. To each of these beakers, in succession, are added the following amounts of decinormal sulfuric acid, viz., one-tenth, three-tenths, six-tenths, one, and one and four-tenths cubic centimeters. After an hour a small quantity of the solution is taken from beaker No. 3 and the reaction of the invertase stopped by adding a few drops of strong potassium hydroxid and the time of adding this reagent noted. This solution is then read in the polariscope and the percentage of sugar inverted is calculated from the formula $C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6$.

The calculation of the amount of cane-sugar inverted is based on the formula, $(38.4-d) \div 0.518 = p$. In this formula d equals the divisions of the sugar scale read on the polariscope; p the percentage of cane-sugar inverted; 38.4 the reading on the sugar scale of the original sugar solution and 51.8 the total number of divisions of the cane-sugar scale that the polariscope reading would fall through if all the sugar were inverted. The observation tubes used in polarization are only 100 millimeters in length. After stopping the action of the invertase with potassium hydroxid the solution is allowed to stand for some time before polarization inasmuch as the dextrose formed appears to assume the state of birotation and some time is required for it to reach its normal rotatory power. If the invertase be used in the

alcoholic solution a sufficient quantity should be added to be equivalent to 0.01 of the sucrose present. The time which the contents of beaker No. 3 will take to reach optical activity is calculated in a manner described by O'Sullivan and Tompson, but too long to be inserted here. The five beakers mentioned above are examined in succession and the amount of sulfuric acid best suited to the maximum inversion thus determined. This quantity is then used in subsequent hydrolyses with the given sample of invertase.

The action of invertase on sucrose is very rapid at the first and becomes very much slower towards the end. At a temperature of 15°.5 it is advisable to let the solution stand for forty-eight hours in order to be sure that complete inversion has taken place.

136. Hudson's Method.—Hudson has devised the following method of preparation of invertase and its use in the estimation of cane-sugar.⁴³ The stock solution of the enzyme may be kept for months and is prepared as follows: Crumble pure compressed yeast by hand and knead it with an equal weight of water at ordinary temperature, saturate the mass with chloroform and keep it at 20° to 30° for forty-eight hours. Add neutral lead acetate to slight excess, filter, remove the excess of lead with potassium oxalate, repeat the filtration, saturate the filtrate with toluene and preserve it in an ice box as the stock solution. The determination of cane-sugar is made by polarization before and after inversion by invertase, using the formula

$$\text{Per cent. cane-sugar} = \frac{S - I}{141.7 - \frac{t}{2}} \cdot 100,$$

where S and I are the direct and invert polarizations of normal weight of the sample, t is the temperature of the invert polarization, and 141.7 is the Clerget factor for invertase inversions, which is slightly less than that for acid inversions, 142.7, because the acid increases the negative rotation of invert sugar. The determination of cane-sugar is carried out as follows: Dissolve 26

⁴³ Hudson, *Journal of Industrial and Engineering Chemistry*, 1910, 2: 143-6.

grams of the substance to be analyzed for cane-sugar in water, clarify with the usual substances (neutral or basic lead acetate, alumina cream or kaolin) and make up to 100 cubic centimeters at 20°. Filter and read the polarization of the filtrate in a 200 millimeter tube. Remove the excess of lead from the filtrate, if lead has been used to clarify, with sodium carbonate or potassium oxalate, and filter. To 50 cubic centimeters of the filtrate add acetic acid by drops until the reaction is just distinctly acid to litmus (since invertase acts only in weakly acid solutions), add five cubic centimeters of the stock invertase solution and make up the volume to 100 cubic centimeters. Add a few drops of toluene to the solution to prevent the growth of micro-organisms, shaking so as to saturate, and allow to stand at any temperature between 20° and 40° over night. Under usual conditions about six hours time is required to accomplish complete hydrolysis. In the morning bring the temperature to 20° and read the rotation of the solution in a 400 millimeter tube. The percentage of cane-sugar present is then calculated by the formula given above. Whether complete inversion has been attained should always be controlled by allowing the solutions to stand and noting their rotations until they reach constancy. The inversion by invertase requires more care than that by acid, and the chief value of the invertase method is in the investigation of new plants and products. If parallel analyses by the acid and invertase methods show different results it is evident that some easily hydrolyzed carbohydrate, other than cane-sugar, is present, and the isolation of the indicated substance in a pure state may then be attempted.

137. Summary.—The enzyme invertase accomplishes a complete inversion of cane-sugar and is without action on starch, dextrin, maltose, lactose, pentosans and natural glucosides. It is found that raffinose is hydrolyzed by invertase and that its specific rotation is thereby reduced from 123° to 67°.6; raffinose accordingly interferes with the estimation of cane-sugar by the use of invertase, just as it also interferes when the inversion is carried out with hydrochloric acid. Analyses of cane-sugar in pure cane-sirup, Louisiana molasses, and bagasse by the invertase

method of hydrolysis give percentages which agree with those obtained by the hydrochloric acid hydrolysis. The Sotol plant (*Dasyllirion texanum*) is shown to contain only a trace of cane-sugar, but 13 per cent. of an unknown carbohydrate which is being further investigated.

138. The Action of Invertase on Other Acid-Hydrolyzable Substances.—Hudson has found invertase to be entirely incapable of hydrolyzing lactose, maltose, starch, dextrin, cellulose, pentosans, amygdalin, or salicin. The trisaccharid raffinose, which occurs in cotton-seed, in wheat, and in the sugar beet, is hydrolyzed by invertase, and the method for estimating cane-sugar by inversion with invertase is accordingly not applicable in case raffinose is present. Raffinose is also hydrolyzed by hydrochloric acid, and therefore the acid hydrolysis has here no advantage over that of invertase. So far no action on any substances except cane-sugar and raffinose has been detected, but it appears probable that the tetrasaccharid stachyose, which occurs in some plants, would be split into fructose and a trisaccharid.

139. Application of the Process.—In practice the process of inversion is used chiefly in the analysis of molasses and low grade massecuites, but it should be used in all check determinations for sucrose. In approximately pure sugars the direct polarization is sufficiently accurate for all practical purposes. In molasses resulting from the manufacture of beet sugar are often found considerable quantities of raffinose, and the inversion process has been adapted to that character of samples. In molasses from cane-sugar factories, the disturbing factors are chiefly invert sugar and gums. Hydrochloric acid changes the rotation of some of the sugars, and also has the power of breaking down some glucosides and thereby causing error in the invert reading. A most notable instance of this is reported by Hudson.⁴⁴

140. The Effect of Alcohol on Invertase.—A knowledge of the action of alcohol on invertase is of practical importance for two reasons; first, alcohol is naturally present during the fermentation of cane-sugar by yeast and the invertase of the yeast is thus normally in the presence of weak alcohol; and, second, alco-

⁴⁴ Bureau of Chemistry, Circular 50, Feb., 1910: 7, 8.

hol is often used, though generally with little success, to prepare the enzyme in a solid form by precipitation from an aqueous extract of yeast. In order to learn the influence of different strengths of alcohols on invertase Hudson and Paine⁴⁵ made a careful investigation. The results show that alcohol destroys invertase, and the relation between alcoholic strength and rate of destruction shows a high maximum at about 50 per cent. alcohol. The destruction proceeds too slowly to be of any effect below 20 per cent. alcohol; at 30° C., is almost instantaneous at 50 per cent., and decreases to nearly zero at 80 per cent. Invertase can therefore be used as an inverting agent in solutions containing as high as 20 per cent. alcohol without danger of its destruction. If the alcohol contains cane-sugar, the destruction is much slower; thus, 6 per cent. cane-sugar reduces the rate of destruction in 50 per cent. alcohol to about 1 per cent. of its original value.

Invertase can be precipitated by alcohol without destruction, provided the strength of alcohol in the final solution is high, approximately 90 per cent. By this method of precipitation, working at room temperature, a solid preparation was obtained which had 78 per cent. of the activity of the original enzyme solution. If cane-sugar is present, invertase can be precipitated with no important destruction by even 70 per cent. alcohol; this method of precipitation gave a recovery of 94 and 96 per cent. of the original activity.

141. Determination of Sucrose and Raffinose.—To determine sucrose and raffinose together the following method is used. The inversion is effected by means of hydrochloric acid and in the manner described by Clerget. The following formulas devised by Herzfeld are calculated for a temperature of observation of 20° and the readings should be made as near that temperature as possible.

$$S = \frac{0.5124 P - I}{0.839}$$

$$R = \frac{P - \text{sucrose}}{1.85}$$

⁴⁵ Bureau of Chemistry, Circular 58, July 30, 1910: 1, 8.

In these formulas S and R are the respective per cents. of sucrose and raffinose described, P the polarization in sugar degrees before inversion, I the polarization after inversion read at 20° . It must be understood that these formulas are applicable only to a solution containing no other optically active substances, save sucrose and raffinose.

142. Specific Rotatory Power.—In order to compare among themselves the rotations produced on a plane of polarized light by different optically active bodies in solution it is convenient to refer them all to an assumed standard. The degree of rotation which the body would show in this condition, is found by calculation, since, in reality, the conditions assumed are never found in practice. In the case of sugars and other optically active bodies, the standard of comparison is called the specific rotatory power. This factor in any given case, is the angular rotation which would be produced by any given substance in a pure anhydrous state if it were one decimeter in length and of a specific gravity equal to water. These are conditions which evidently do not exist in the case of sugars, since crystalline sugar particles have no polarizing power, and it would be impossible to pass a ray of light through an amorphous sugar column of the length specified. The specific rotatory power is therefore to be regarded as a purely theoretical factor, calculated from the actual data obtained by the examination of the solution of any given substance.

143. Formulas for Calculating Specific Rotatory Power.—In order to determine the specific rotatory power (gyrodyn⁴⁶) of a given substance it is necessary to know the specific gravity and percentage composition or concentration of its solution, and to examine it with monochromatic polarized light in an instrument by which the angular rotation can be measured. The ordinary saccharimeters may also be used but in such cases the readings of the sugar scale must be calculated to circular degrees by the formulas already given. The gyrodyn of any body changes with its degree of concentration, in some cases with the

⁴⁶ Abbreviated from *gyrodynat*, ($\gamma\rho\omega\acute{\alpha}$, $\delta\rho\nu\alpha\mu\acute{o}\varsigma$) = specific rotatory power.

temperature, and always with the color of the light. With the red rays the rotation is least and it progressively increases as the violet end of the spectrum is approached. In practice the yellow ray of the spectrum has been found most convenient for use, and in the case of sugars the gyrodyn is always expressed either in terms of this ray or if made with color compensating instruments in terms of the sensitive or transition tint. In the one case the symbol used is $(a)_D$ and in the other $(a)_J$. From this statement it follows that $(a)_D$ is always numerically less than $(a)_J$. Unless otherwise specified the gyrodyn of a body is to be considered as determined by yellow monochromatic light, and therefore corresponds to $(a)_D$.⁴⁷ A figure given after D or J is the temperature at which the rotation was taken. If none is given 20° is the one meant.

If the length of the observation tube in decimeters be represented by l , the percentage of the polarizing body in 100 grams by p , and the specific gravity of the solution by d , and the observed angle of rotation by a , then the factor is calculated from the formula:

$$[a]_D \text{ or } J = \frac{a \cdot 100}{p \cdot d \cdot l}.$$

The symbols D or J refer to the character of light employed, D indicating the monochromatic sodium flame, and J the transition tint from white light. The length of the wave J is not definitely determined but its value is generally recognized as 550 micromillimeters while the value of the D line is 589. The following formulae are used for converting one expression into the other.⁴⁸

$$(a)_J = 1.128 (a)_D$$

$$(a)_D = 0.887 (a)_J.$$

If the weight of the polarizing body c be given or known for 100 cubic centimeters of the solution the formula becomes

$$[a]_D \text{ or } J = \frac{a \cdot 100}{c \cdot l}.$$

⁴⁷ Landolt, *Handbook of the Polariscopes*, 1882: 225, 48.

⁴⁸ Landolt, *Handbook of the Polariscopes*, 1882: 46.

The latter formula is the one easier of application since it is only necessary in applying it to dissolve a given weight of the active body in an appropriate solvent and to complete the volume of the solution exactly to 100 cubic centimeters. It is therefore unnecessary in this case to determine the specific gravity.

144. Variations in Specific Rotatory Power.—The gyrodyn of any optically active body, the source of illumination remaining constant, varies with the nature of the solvent, the strength of the solution and the temperature.

Since water is the only solvent of importance in determining the gyrodyn of sugars it will be necessary here to discuss the influence of the nature of the solvent. In respect to the strength of the solution it has been established that in the case of cane-sugar the gyrodyn decreases while with dextrose it increases with the degree of concentration. The influence of temperature on the rotation of common sugars is not of great importance save in the case of levulose, where it is the most important factor, the rotation rapidly increasing as the temperature falls. It is of course understood that the above remarks do not apply to the increase or decrease in the volume of a solution at changed temperatures. This influence of temperature is universally proportional to the change of volume in all cases, and this volumetric change is completely eliminated when the polarizations are made at the temperatures at which the solutions are completed to standard volumes.

145. Gyrodyn of Sucrose.—In the case of cane-sugar the gyrodyn for 25 grams of sugar in 100 grams of solution at 20° is $[\alpha]_D = 66^\circ.37$. This is about the degree of concentration of the solutions employed in the shadow lamp-light polariscopes. For 17 grams of sugar in 100 grams of solution the number is $[\alpha]_D = 66^\circ.49$. This is approximately the degree of concentration for the laurent instrument.

For any degree of concentration according to Tollens the gyrodyn may be computed by the following formula: $[\alpha]_D = 66^\circ.386 + 0.015035p - 0.0003986p^2$, in which p is the number of

grams of sugar in 100 grams of the solution.⁴⁹ In the table constructed by Schmidt the data obtained are as follows:

In 100 parts by weight of solution. Sugar <i>g.</i> Water <i>g.</i>		Specific gravity at 20° C. <i>d.</i>	Concentration <i>c</i> in <i>g./l.</i>	Rotation α for 100 mm. at 20° C.	$[\alpha]_D$
64.9775	35.0225	1.31650	85.5432	56°.134	65°.620
54.9643	45.0357	1.25732	69.1076	45°.533	65°.919
39.9777	60.0223	1.17664	47.0392	31°.174	66°.272
25.0019	74.9981	1.10367	27.5938	18°.335	66°.441
16.9946	83.0054	1.06777	18.1442	12°.064	66°.488
9.9997	90.0003	1.03820	10.3817	6°.912	66°.574
4.9975	95.0025	1.01787	5.0868	3°.388	66°.609
1.9986	98.0014	1.00607	2.0107	1°.343	66°.802

146. Mutarotation (Biorotation and Multirotation).—Some sugars have the property of showing a higher or lower rotatory power when first dissolved than afterwards. This phenomenon is called mutarotation and was discovered by Dubrunfaut in 1846. He observed that the freshly prepared solution in cold water of dextrose, has an initial value of about 110°, but gradually loses this rotatory power and becomes constant at about 52°. Hudson has reviewed the literature of mutarotation and brought the subject up to the latest expressed theories.⁵⁰

Many crystalline sugars exhibit the phenomenon of mutarotation, namely: lactose, galactose, arabinose, maltose, xylose, levulose, fucose, rhamnose, mannose, rhodose, gentiobiose, melibiose, perseulose and a few synthetic sugars. All sugars which show mutarotation reduce alkaline copper solution and form compounds with phenylhydrazin. This shows that they belong to the classes aldoses or ketoses, and contain the carbonyl group. Sugars which do not contain the carbonyl group do not exhibit mutarotation. Such sugars are sucrose, raffinose, gentianose and stachyose. The starches, inulin and mannan and certain glucosides, such as salicin, amygdalin, helicin, and arbutin do not contain the carbonyl group and do not show any mutarotation. From these facts it is reasonable to infer that mutarotation is dependent upon the carbonyl group.

⁴⁹ *Berichte der deutschen chemischen Gesellschaft*, 1877, 10: 1403.

⁵⁰ *Journal of the American Chemical Society*, 1910, 32: 889.

Hudson has developed the idea advanced by Lippmann that the lactonic formula for dextrose affords two possible forms of the sugar on account of the asymmetry of the end carbon atom. Hudson concludes that the mutarotation reaction is general to all the aldehyde and ketone sugars and it may be considered the fundamental reaction of the sugar group. It is evident, therefore, that the application of the theories based on these facts will prove extremely fruitful in explaining the chemical and biological relations of the sugars.

After standing for a few hours, or immediately on heating, solutions of sugars that show mutarotation assume a normal state of equilibrium. The addition of a small quantity of an alkali, such as ammonia, also causes the disappearance of the mutarotation, either instantly or in a very short time.

C. A. Browne has recently discussed the optical rotatory powers of the sugars from the standpoint of the theory of electrons. According to this theory the valence between atoms has a definite direction. After assigning the directions of the valencies between the atoms which compose the molecules of the various sugars Browne finds that there is usually a parallelism between the rotatory power of the sugar and the predominating valence direction. According to Browne the valence directions change slowly when a crystalline sugar is dissolved, and this change causes the phenomenon of mutarotation unpublished.

147. Gyrodyne of Dextrose.—The gyrodyne of dextrose, as has already been mentioned, increases with the degree of concentration, thus showing a property directly opposite to that of sucrose.

The general formula for the anhydrous sugar is $[\alpha]_D = 52.718 + 0.017087p + 0.0004271p^2$. In this formula p represents the grams of dextrose in 100 grams of the solution. In a ten per cent. solution the gyrodyne of dextrose is therefore nearly exactly $[\alpha]_{D20} = 53^\circ$. As calculated by Tollens the gyrodynes corresponding to several degrees of concentration are shown in the following table:

p = grams in 100 grams of solution.	$[\alpha]_D^{20}$ calculated for anhydrous dextrose.
7.6819	$52^\circ.89$
92.904	$52^\circ.94$
9.3712	$52^\circ.94$
10.0614	$52^\circ.96$
10.6279	$52^\circ.98$
12.9508	$53^\circ.05$
18.6212	$53^\circ.25$
31.6139	$53^\circ.83$
40.7432	$54^\circ.34$
43.9883	$54^\circ.54$
53.0231	$55^\circ.17$
82.6111	$57^\circ.80$

148. Rotations of Other Sugars.—Of the other sugars it will be sufficient to mention only levulose, maltose, lactose, and raffinose.

The gyrodyn of levulose is not definitely established. At 14° the number is nearly expressed by $[\alpha]_D^{14} = -93^\circ.7$.

Invert sugar, which should consist of exactly equal molecules of dextrose and levulose, has a gyrodyn expressed by the formula $[\alpha]_D^{20} = -27^\circ.9$, with a concentration equivalent to 17.21 grams of sugar in 100 cubic centimeters. The gyrodyn decreases with increase of temperature, according to the formula $[\alpha]_D^t = -(27^\circ.9, -0.32 t^\circ)$. According to this formula the solution is neutral to polarized light at $87^\circ.2$, and this corresponds closely to the data of experiment.

Maltose, in a 10 per cent. solution at 20° , shows a gyrodyn of $[\alpha]_D^{20} = 138^\circ.3$.

The general formula for other degrees of concentration is $[\alpha]_D = 140^\circ.375 - 0.01837 p - 0.095 t$, in which p represents the number of grams in 100 grams of the solution and t the temperature of observation.

In the case of lactose $[\alpha]_D = 52^\circ.53$, and this number does not appear to be greatly influenced by the degree of concentration, but is somewhat diminished by a rising temperature.

The gyrodyn of raffinose in a 10 per cent. solution is $[\alpha]_D = 104^\circ.5$.

CHEMICAL METHODS OF ESTIMATING SUGARS.

149. General Principles.—The methods for the chemical estimation of sugars in common use depend on the reducing actions exerted on certain metallic salts, whereby the metal itself or some oxid thereof, is obtained. The reaction is carried on either volumetrically or the resulting oxid or metal may be weighed or titrated. The common method is, therefore, resolved into two distinct processes, and each of these is carried out in several ways. Not all sugars have the faculty of exerting a reducing action on highly oxidized metallic salts. In general, all monosaccharides having an aldehydic or ketonic function do show this power, while in the disaccharides not all reduce solutions of metal salts; only those in which the aldehydic or ketonic group of the second hexose remains functional being able to reduce such solution. These latter disaccharides, however, by simple hydrolysis become reducing. In all cases the reducing power of a sugar solution is largely dependent on its degree of concentration, and this factor must always be taken into consideration. Salts of copper and mercury are most usually selected to measure the reducing power of a sugar and in point of fact copper salts are almost universally used. Copper sulfate and carbonate are the salts usually employed, and of these the sulfate far more frequently, but only after conversion into tartrate. Practically therefore, the study of the reducing action of sugar as an analytical method will be confined almost exclusively to the determination of its action on copper tartrate.

Direct gravimetric methods are also practiced to a limited extent in the determination of sugars as in the use of the formation of sucrates of the alkaline earths and of the combinations which certain sugars form with phenylhydrazin. This last named reaction has assumed a marked degree of importance as an analytical method. The most practical treatment of this section, therefore, for the limited space which can be given it, will be the study of the reducing action of sugars, both from a volumetric and gravimetric point of view, followed by a description of the best approved methods of the direct precipitation of sugars

by such reagents as barium hydroxid and phenylhydrazin and its many substitution products.

VOLUMETRIC REDUCING METHODS.

150 Classification.—Among the volumetric methods will be given those which are in common use or such as have been approved by the practice of analysts. Since the use of mercuric salts is now practiced to a very limited extent, only a brief study of that process will be attempted. With the copper methods a somewhat extended description will be given of those depending on the use of copper sulfate, and a briefer account of the copper carbonate process.

In the copper sulfate method two distinct divisions must be noted, *viz.*, first an indirect process depending first upon the reduction of the copper to a suboxid, the subsequent action of this body on iron salts, measured finally by titration with potassium permanganate as an indirect method of titration of the copper; and second, a direct process determined either by the disappearance of the blue color from the copper solution, or by the absence of copper from a drop of the solution withdrawn and tested with potassium ferrocyanid. This last mentioned reaction is one which is found in common use. The volumetric methods are not, as a rule, as accurate as the gravimetric, depending on weighing the resultant metal, but they are far more rapid and well suited to technical control determinations.

151. Reduction of Mercuric Salts.—The method of determining sugar by its action on mercuric salts, is due to Knapp.⁵¹ The method is based on the observation that dextrose and other allied sugars, will reduce an alkaline solution of mercuric cyanid, and that the mercury will appear in a metallic state.

The mercuric liquor is prepared by adding to a solution of 10 grams of mercuric cyanid, 100 cubic centimeters of a solution of caustic soda of 1.145 specific gravity, and making the volume

⁵¹ *Annalen der Chemie und Pharmacie*, 1870, **154**:252.

to one liter with water. The solution of sugar to be titrated, should be as nearly as possible of one per cent. strength.

To 100 cubic centimeters of the boiling solution, the sugar solution is added in small portions from a burette and in such a way as to keep the whole mass in gentle ebullition.

To determine when all the mercuric salt has been decomposed, a drop of the clear boiling liquid is removed and brought into contact with a drop of stannous chlorid solution on a white surface. A brownish black coloration or precipitate will indicate that the mercury is not all precipitated. Fresh portions of the sugar must then be added, until no further indication of the presence of mercury is noted. The approximate quantity of sugar solution required to precipitate the mercury having thus been determined, the process is repeated by adding rapidly, nearly the quantity of sugar solution required, and then only a few drops at a time, until the reduction is complete.

One hundred cubic centimeters of the mercuric cyanid solution prepared as directed above, will be completely reduced by

202	milligrams	of dextrose.
200	"	" invert sugar.
198	"	" levulose.
308	"	" maltose.
311	"	" lactose.

By reason of the unpleasant odor of the boiling mercuric cyanid when in presence of a reducing agent, the process should be conducted in a well-ventilated fume chamber. With a little practice the process is capable of rapid execution, and gives reasonably accurate results.

152. Sachsse's Solution.—The solution of mercuric salts proposed by Sachsse is made by dissolving 18 grams of mercuric iodid in 25 cubic centimeters of an aqueous solution of potassium iodid. To this solution are added 200 cubic centimeters of potash lye, containing 80 grams of caustic potash. After mixing the solution, the volume is completed to one liter. The sugar solutions used to reduce this mixture, should

be more dilute than those employed with the mercuric cyanid, and should not be over one-half per cent in strength. The end of the reduction is determined as already described. After a preliminary trial, nearly all the sugar necessary to complete reduction, should be added at once, and the end of the reduction then determined by the addition of successive small quantities. One hundred cubic centimeters of the mercuric iodid solution prepared as directed above, require the following quantities of sugar to effect a complete reduction:

325	milligrams of dextrose.
269	" " invert sugar.
213	" " levulose.
491	" " maltose.
387	" " lactose.

By reason of the great difference between the reducing power of dextrose and levulose in this solution, it has been used in combination with the copper reduction method, to be described, to determine the relative proportion of dextrose and levulose in a mixture.⁵²

Instead of using stannous chlorid as an indicator, the end of the reaction may be determined as follows: A disk of filtering paper is placed over a small beaker containing some ammonium sulfid. A drop of the clear hot solution is placed on this disk, and if salts of mercury be still present a dark stain will be produced; or a drop of the ammonium sulfid may be brought near the moist spot formed by the drop of mercury salt. An alkaline solution of zinc oxid may also be used.

The methods depending on the use of mercuric salts have, of late, been supplanted by better processes, and space will not be given here to their further discussion.

153. The Volumetric Copper Methods.—The general principle on which these methods depend, is found in the fact that certain sugars, notably, dextrose, (glucose), levulose, (fructose), maltose and lactose, have the property of reducing an alkaline solution of cupric salts to cuprous salts. (Tucker, *Manual of Sugar Analysis*; 1883. 208.

tion of copper to a lower state of combination, in which the copper is separated as cuprous oxid. The end of the reaction is either determined by the disappearance of the blue color of the solution, or by the reaction produced by a drop of the hot filtered solution, when placed in contact with a drop of potassium ferricyanid treated with a slight excess of acetic acid.

The copper salt which is found to give the most delicate and reliable reaction, is the tartrate. The number of volumetric processes proposed and which are in use, is very great, and an attempt even to enumerate all of these can not be made in this volume. A few of the most reliable and best attested methods will be given, representing if possible, the best practice in this and other countries. The rate of reduction of the copper salt to suboxid, is influenced by the rate of mixing with the sugar solutions, the temperature, the composition of the copper solution and the strength of the sugar solution.

The degree of reduction is also modified by the rate at which the sugar solution is added, and by the degree and duration of heating, and all these variables together, make the volumetric methods somewhat difficult and their data, to a certain extent, discordant. By reason, however, of the ease with which they are applied and the speed of their execution, they are invaluable for approximately correct work and for use in technical control.

145. Fehling's Solution.—*A contribution to the history of chemical reagents.*⁵³ A history of chemical reagents would form no small and not the least interesting part of the history of chemistry generally, and for obvious reasons. In studying the intramolecular changes of matter, chemistry follows strictly inductive methods, taking for granted only the results of direct observation, and while for the purpose of unification we are compelled to classify such results into some comprehensive and consistent system arrived at by logical induction, such hypothetical assumptions are not permitted to influence the direct results of the experiments.

⁵³ Herstein, *Journal Chemical Society*, 1910, **32**:779.

Intramolecular changes of matter however are not always directly observable, and to bring them into a condition where our perception would be able to grasp them, we must in the majority of cases resort to what may be described as secondary reactions induced by foreign substances, the so-called chemical reagents. Our processes of differentiation, upon which chemistry in general and analytical chemistry especially ultimately rest, are based upon the behavior of the various forms of matter towards different chemical reagents, and it follows from this that the progress of chemistry to a very large degree depends upon the introduction of such chemical reagents and an intimate knowledge of their working.

Thus to quote but one modern instance it is only necessary to point to the fact that the wonderful insight which we have gained within the last generation into the nature of sugars and carbohydrates generally is due almost exclusively to the introduction of phenylhydrazin as a chemical reagent. Previous to this our knowledge of this group of substances was very limited indeed, and of the few reactions characteristic for this group of substances none was better known than their behavior towards a solution of copper salts. The reaction upon which the value of this reagent in all its various modifications depends is, as is well known, based upon the reducing effect which certain sugars exert upon such solution, and although analytical chemistry has only within comparatively recent years made use of this reaction, its history runs nevertheless far back into the primitive period of human knowledge.

It undoubtedly must first have been observed by the man who introduced the so-called *unguentum Aegyptiacum* Egyptian ointment—into materia medica, and this probably was none other than an Egyptian priest, if we may draw such conclusions from the name. This *unguentum Aegyptiacum*, which has long since disappeared from all the modern pharmacopoeias, but which for long ages was considered a very valuable medicine, forming the basis of various ointments and cosmetrical preparations, was prepared by boiling together a mixture of verdigris and honey

to which a few drops of vinegar were added. During the boiling the color of the mass changes considerably owing to the interaction between the glucose and the copper acetate, a change of color so striking that it could not have escaped notice. Nevertheless it has never occurred to any of these pharmacists, who in those days were the principal guardians of whatever chemistry there was, to make these changes the independent object of their studies. The first to attempt an explanation of the phenomena observed during this reaction was Antoine Baumé, a celebrated pharmacist of the eighteenth century, who wrote his name indelibly into the history of chemical technology by the device of his spindle. In accordance with the theory then prevailing he said in his "*Traité de Pharmacie Théorique et Pratique*," that in boiling honey with verdigris and vinegar it is the phlogiston of the acetic acid which, uniting with the verdigris, forms metallic copper, which is deposited. For almost one-half a century this explanation was accepted as satisfactory. During this time, however, the phlogiston theory had been entirely overthrown by Lavoisier, and a period of reconstruction had set in, which may be described by Hegel's phrase "the re-evaluation of all values." Speculative deductions gave place to experimental research, and the balance was made the chief arbiter of the laboratory. A thorough overhauling of all chemical knowledge was undertaken, and in course of time the phenomena observed during the reaction in question were also subjected to renewed scrutiny.

In January, 1815, Dr. Vogel, of Paris, read before the French Academy of Science a paper entitled "A Research Concerning the Decomposition of Salts and Metallic Oxides by Sugars."⁵⁴ As indicated by the title, the principal object sought in this investigation was an insight into the mechanism of the reaction, while the products formed during the reaction were studied as closely as the means then permitted, both qualitatively and quantitatively, but only with a view of obtaining light upon the reaction itself. Vogel was able to show that the reddish or

⁵⁴ Abstracted in Schweigger's Journal, 1815, 13 : 162.

brown precipitate which formed on boiling copper acetate with honey and several other sugars then known was not metallic copper, but cuprous oxide, or as it was then called, copper protoxid, that the color of the precipitate depended very much on the length of time the mixture was heated, and that the precipitation is obtained only from cane, starch and grape sugar, sugar of milk, and from molasses, manna, and honey, but not from the so-called sweet principle of Scheele.⁵⁵ Vogel also observed the evolution of carbonic acid gas and acetic acid, which, however, he attributed to the high temperature to which the mixture had been subjected: Using copper sulfate instead of the acetate he claims to have precipitated metallic copper while copper chlorid and copper nitrate, which were likewise tried, seemed not to be influenced at all. Similar observations made almost simultaneously with the preceding were given by J. A. Buchner, Professor of Chemistry in Munich, and published in the succeeding number of the same periodical.⁵⁶ On the whole he merely confirmed the result obtained by Vogel with this exception, that while Vogel had proved the red precipitate to consist of cuprous oxide, Buchner states that during the reaction the sugar is split up and that one of these cleavage products combines with the copper to form the precipitate.

From time to time other chemists were attracted by the same subject.⁵⁷ Their work, however, did not materially broaden the problem, which remained one more of theoretical than practical value until the year 1841, when Trommer published his paper, "The Differentiation between Dextrin, Cane and Grape Sugar."⁵⁸ Here the subject was approached from an entirely different and more practical point of view. Trommer was able to show that in making the copper salt solution alkaline, it became a valuable reagent for the differentiation between the various sugars and

⁵⁵ By this name glycerol, discovered in 1779 by Scheele, was known until well up into the first part of the 19th century.

⁵⁶ Schweigger's Journal, 1815, 15 : 224.

⁵⁷ Buchholtz, Peschier, Busch, see *Annalen der Chem. und Pharm.*, 1832, 157.

⁵⁸ *Annalen der Chem. und Pharm.*, 1841, 39 : 360.

sugar giving substances. He used an alkaline solution of copper sulfate, and showed that such a solution will yet give a distinctive precipitate of cuprous oxid when boiled with solutions of grape sugar containing only one part in one hundred thousand, and that when the solution of the grape sugar is ten times more dilute, *i.e.*, containing one part to one million, the reaction is yet noticeable in reflected light. The fact that the reaction takes place only in the presence of certain sugars such as glucose, for instance, while cane-sugar or dextrin are not acted upon, was fully noticed and made the basis of the differentiation between the different sugars. To Trommer thus belongs the credit of having first introduced the alkaline copper sulfate solution into analytical chemistry, though it was at first used only for qualitative purposes. But the next step was not long in coming, and within a very short time the very same reagent was recommended for use in quantitative chemical analysis.

The estimation of sugars in those days was far from being satisfactory to the chemist engaged in this line of work, depending entirely on two methods. First, the fermentation process devised by Lavoisier and others, and second, the process introduced by Biot, which was based on the deflection of polarized light by sugar solutions. Both methods have their disadvantages, the first requiring considerable time for its completion, quite aside from the fact that the decomposition into alcohol and carbonic acid gas is by no means quantitative, by-products being formed which interfere with the accuracy of the results. The polariscope gives quick and fairly accurate results; requires however clear and colorless solutions containing no foreign substances which would deflect the polarized ray. The necessity for an improved method of estimating sugars became so imperious that in 1838 a prize of 3,000 francs was offered by the "Société d'encouragement pour l'industrie nationale" to the chemist who would devise a successful method for the quantitative estimation of sugar. Of the answers received none was acceptable to the committee, and the offer was renewed from year to year until in 1844 Ch. Barreswil submitted his solu-

tion.⁵⁹ The referee of the committee, the well-known Peligot, did not consider the answer entirely satisfactory, but he nevertheless recommended that one-third of the prize, viz., 1,000 francs, be awarded to Barreswil for his solution, which, in his opinion, though limited in its application, nevertheless offered a valuable improvement on the existing methods. Barreswil's proposal consisted merely in the extension of the method suggested by Trommer for qualitative differentiation to the quantitative estimation of sugars. The main principle of Trommer's suggestion, i.e., the use of the alkaline copper sulfate solution, was maintained, and the improvement introduced consisted in the addition of a solution of potassium tartrate to prevent the decomposition of the solution on heating. As proposed by Barreswil, the method was a volumetric one, the copper solution being standardized against pure grape sugar solution of known strength, which latter was added drop by drop to the boiling copper solution, the end point of the reaction being reached when the blue color of the solution had disappeared. He also points to the necessity of inverting the cane-sugar to glucose, and making two determinations, before and after inversion, where mixtures of sugars are to be analyzed.

The principle of the method was thus established, and its practicability depended very largely upon the stability of the copper solution, which was not very satisfactory. The further efforts of the chemists who studied the subject were therefore directed mainly in this direction although the use of the method was fully recognized and extended, as by H. Schwartz,⁶⁰ who in 1849 proposed it for the estimation of starch, which had previously to be hydrolyzed by dilute sulfuric acid to glucose. H. Fehling, by whose name the method is apparently destined to remain known in chemistry, published his first paper concerning the subject in 1848 in a medical publication, and only in 1849 he gave a more detailed account of his work to the chemical fraternity.⁶¹ Feh-

⁵⁹ *Journal de Pharmacie* [3], 1844, 6 : 301.

⁶⁰ *Ann.*, 1849, 70 : 54.

⁶¹ *Ibid.*, 1849, 62 : 106; 1858, 106 : 75.

ing's merit consisted chiefly in having worked out with great care the details of the method, giving some account of the stoichiometrical equivalents, but neither did his solution keep for any length of time, nor did he, any more than his predecessors, recognize the fact that the reaction is quantitative only within very narrow restrictions as to the concentration of the solutions and the time of reaction. This point has been established only by the concerted efforts of the many chemists, who worked on the subject after Fehling, the necessity for keeping the copper solution divided into two parts being soon recognized. Soxhlet especially devoted much of his time to the study of the method and established⁶² the exact conditions under which the determination must be carried out in order to get satisfactory results.

Since the date mentioned the principal improvements in the process have been in changing the composition of the copper solution in order to render it more stable, and to reduce its action on sucrose which has been accomplished by varying the proportions of copper sulfate, alkali and tartaric acid. For the better keeping of the solution the method of preserving the copper sulfate and the alkaline tartrates in separate flasks and only mixing them at the time of use has been found very efficacious and is used now altogether.⁶³ For testing for the end of the reaction by means of an acetic acid solution of potassium ferrocyanid the filtering tube suggested by the author, the use of which will be described further on, has proved quite useful. Pavy has suggested that by the addition of ammonia to the copper solution the precipitated suboxid may be kept in solution and the end of the reaction thus easily distinguished by the disappearance of the blue color.⁶⁴ Allen has improved on this method by covering the hot mixture with a layer of paraffin oil whereby any oxidation of the suboxid is prevented.⁶⁵

⁶² *Z. anal. Chem.*, **18**; **20**: 425. For other references to modern investigators see standard text-books.

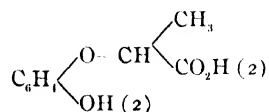
⁶³ Rodewald and Tollens, *Berichte der deutschen chemischen Gesellschaft*, B. **11**, 2076.

⁶⁴ *Chemical News*, **39**: 77.

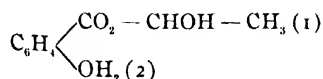
⁶⁵ *The Analyst*, **19**: 181.

The introduction and development of the gravimetric process depending on weighing the reduced copper either as cuprous or cupric oxid in a metallic state as developed by Allihn, Soxhlet, Munson and Walker and others, completes the resumé of this brief sketch of the rise and development of the process.

155. Action of Alkaline Copper Solution on Dextrose.—The action to which dextrose and other reducing sugars are subjected in the presence of a hot alkaline copper solution is two-fold in its nature. In the first place there is an oxidation of the sugar which is transformed into tartronic, formic and oxalic acids, the two latter in very small quantities. At the same time another part of the sugar is attacked directly by the alkali and changed to complex products among which have been detected lactic, oxyphenic and oxalic acids, also two bodies isomeric with di-oxyphenolpropionic acid. When the sugar is in large excess melassic and glucic acids have also been detected. The glucic acid may be regarded as being formed by simple dehydration but becomes at once resolved into pyrocatechin and gluconic acid according to the reaction $C_{12}H_{18}O_6 = C_6H_6O_2 + C_5H_{12}O_7$. The gluconic acid also is decomposed and gives birth to lactic and glyceric acids according to the formula $C_6H_{12}O_7 = C_3H_6O_3 + C_3H_6O_4$. The glyceric acid also in the presence of a base is changed into lactic and oxalic acids. Between lactic acid and pyrocatechin, existing in a free state, there is produced a double reciprocal etherification in virtue of which there arise two ethers isomeric with hydrocaffeic acid, $C_6H_{10}O_4$. One of these bodies is an acid and corresponds to the constitution



and the other is of an alcoholic nature corresponding to the formula,



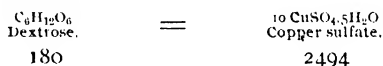
Of all these products only oxyphenic and lactic acids and their ethers and oxalic acid remain unchanged and they can be isolated. All the others are transformed in an acid state and they can only be detected by operating in the presence of metallic oxids capable of precipitating them at the time of their formation.⁶⁶

156. Fehling's Solution.—The solution is originally proposed by Fehling* has the following composition:

Pure crystallized copper sulfate,	40.00 grams :
Neutral potassium tartrate,	160.00 grams :
Sodium hydroxid, (Sp Gr. 1.12)	600 to 700 grams :

The copper sulfate is dissolved in about 160 grams of water and the potassium tartrate in the aqueous solution of the sodium hydroxid which should have a volume of about 700 cubic centimeters. The two solutions are mixed and the volume completed to 1,154.4 cubic centimeters at 15° C. Each cubic centimeter of this solution will be reduced by five milligrams of dextrose, equivalent to four and a half milligrams of sucrose.

The reaction which takes place is represented by the following molecular proportions:



This Fehling solution is delicate in its reactions but does not keep well, depositing cuprous oxid on standing especially in a warm place exposed to light. For this reason its use in the above form has been discontinued. It has been cited by reason of its historic value. Most of the various modifications proposed still bear the name and apparently continue to do so. The Fehling liquor was soon modified in its constitution by substituting 173 grams of the double sodium and potassium tartrate for the neutral potassium tartrate.

157. Modification of Copper Solution.—For the convenience of analysts there is given below a tabular comparison of a few of

⁶⁶ Gaud., Bulletin de l'Association des Chimistes de Sucrerie et Distillerie, 1895, 12:639; Comptes rendus, 1894, 119:604.

* Justus Liebig's Annalen der Chemie und Pharmacie, 1849, 72:111-114.

the different forms of fehling liquor which have been proposed for oxidizing sugars. The table is based on a similar one prepared by Tollens and Rodewald, amended and completed by Horton.⁶⁷ The solutions are arranged alphabetically according to author's names:

1. *Allihn*:

34.6 grams copper sulfate, solution made up to half a liter; 173 grams potassium-sodium tartrate; 125 grams potassium hydroxid (equivalent to 89.2 grams sodium hydroxid), solution made up to half a liter.

2. *A. H. Allen*:

34.64 grams copper sulfate, solution made up to 500 cubic centimeters; 180 grams potassium-sodium tartrate; 70 grams sodium hydroxid (not less than 97% NaOH), solution made up to half a liter.

3. *Bödeker*:

34.65 grams copper sulfate; 173 grams potassium-sodium tartrate; 480 cubic centimeters sodium hydroxid solution, 1.14 specific gravity; (67.3 grams sodium hydroxid); fill to one liter; 0.180 gram dextrose reduces according to Bödeker, 36.1 cubic centimeters of the copper solution = 0.397 gram copper oxid. The same quantity of lactose reduces, however, only 27 cubic centimeters copper solution = 0.298 gram copper oxid.

4. *Boussingault*:

40 grams copper sulfate; 160 grams potassium tartrate; 130 grams sodium hydroxid, made up to one liter.

5. *Dietzsch*:

34.65 grams copper sulfate; 150 grams potassium-sodium tartrate; 250 grams sodium hydroxid solution, 1.20 specific gravity; 150 grams glycerol, made up to one liter.

6. *Fleischer*:

69.28 grams copper sulfate dissolved in about half a liter of water, add to this 200 grams tartaric acid; fill to one liter with concentrated sodium hydroxid solution; 20 cubic centimeters copper solution = 40 cubic centimeters sugar solution, that contain in every cubic centimeter five milligrams dextrose.

7. *Gorup-Besanez*:

34.65 grams copper sulfate; 173 grams potassium-sodium tartrate; 480 cubic centimeters sodium hydroxid solution, 1.14 specific gravity; equal 67.3 grams sodium hydroxid. Fill to one liter.

⁶⁷ Journal of Analytical and Applied Chemistry, 1890, 4: 370.

8. *Grimaux*:

40 grams copper sulfate; 160 grams potassium-sodium tartrate; 600-700 cubic centimeters sodium hydroxid solution, 1.20 specific gravity, equal to 92.5-107.9 grams sodium hydroxid. Fill to 1154.4 cubic centimeters. Ten cubic centimeters of this solution are completely decolorized by 0.050 gram glucose.

9. *Holdfleis*:

34.632 grams copper sulfate in one liter of water; 125 grams potassium hydroxid, equivalent to 89.2 grams sodium hydroxid; 173 grams potassium-sodium tartrate. Fill to one liter.

10. *Hoppe-Seyler*:

34.65 grams copper sulfate; 173 grams potassium-sodium tartrate; 600-700 cubic centimeters sodium hydroxid solution, 1.12 specific gravity; equal to 63.0-73.5 grams potassium hydroxide. Fill to one liter. One cubic centimeter is reduced by exactly 0.005 gram dextrose.

11. *Krocker*:

6.28 grams copper sulfate; 34.6 grams potassium-sodium tartrate; 100 cubic centimeters sodium hydroxid solution, 1.14 specific gravity. Fill to 200 cubic centimeters. In 100 cubic centimeters of this solution is contained 0.314 gram copper sulfate, which is reduced by 0.050 gram dextrose.

12. *Liebermann*:

4 grams copper sulfate; 20 grams potassium-sodium tartrate; 70 grams sodium hydroxid solution, 1.12 specific gravity. Fill to 115.5 cubic centimeters.

13. *Löwe*:

15 grams copper sulfate; 60 grams glycerol; 80 cubic centimeters sodium hydroxid, 1.34 specific gravity; 160 cubic centimeters water. Fill to half a liter.

14. *Mohr*:

34.64 grams copper sulfate; 150 grams di-potassium tartrate; 600-700 cubic centimeters sodium hydroxid solution, 1.12 specific gravity, equal to 70.5-82.3 grams sodium hydroxid. Fill to one liter.

15. *Märcker*:

35 grams copper sulfate, solution made up to one liter; 175 grams potassium-sodium tartrate; 125 grams potassium hydroxid, equivalent to 89.2 grams sodium hydroxid, solution made up to one liter.

16. *Maumené*:

375 grams copper sulfate; 188 grams potassium-sodium tartrate; 166 grams potassium hydroxid. Fill to nine liters.

17. *Monier:*

40 grams copper sulfate; 3 grams stannic chlorid; 80 grams cream of tartar; 130 grams sodium hydroxid. Fill to one liter.

18. *Neubauer and Vogel:*

34.639 grams copper sulfate; 173 grams potassium-sodium tartrate; 500-600 grams sodium hydroxid solution, 1.12 specific gravity. Fill to one liter.

19. *Pasteur:*

40 grams copper sulfate; 105 grams tartaric acid; 80 grams potassium hydroxid 130 grams sodium hydroxid. Fill to one liter.

20. *Possot:*

40 grams copper sulfate; 300 grams potassium-sodium tartrate; 29 grams sodium hydroxid; 159 grams sodium bicarbonate; allow to stand six months before use. Fill to one liter. One cubic centimeter equals 0.0577 gram dextrose. One cubic centimeter equals 0.0548 gram cane sugar.

21. *Rüth:*

34.64 grams copper sulfate; 143 grams potassium-sodium tartrate; 600-700 cubic centimeters sodium hydroxid solution, 1.12 specific gravity. Fill to one liter.

22. *Rodewald and Tollens:*

34.639 grams copper sulfate, solution made up to half a liter; 173 grams potassium-sodium tartrate; 60 grams sodium hydroxid, solution made up to half a liter.

23. *Schorlemmer:*

34.64 grams copper sulfate; 200 grams potassium-sodium tartrate; 600-700 cubic centimeters sodium hydroxid solution, 1.20 specific gravity. Fill to one liter.

24. *Soxhlet:*

34.639 grams copper sulfate, solution made up to half a liter. 173 grams potassium-sodium tartrate; 51.6 grams sodium hydroxid, solution made up to half a liter.

25. *Soldaini:*

34.64 grams copper sulfate; 297 grams potassium bicarbonate. Fill to one liter.

26. *Violette:*

34.64 grams copper sulfate; 187.0 grams potassium-sodium tartrate; 78.0 grams sodium hydroxid made up to one liter. Ten cubic centimeters equal 0.050 gram dextrose. Ten cubic centimeters equal 0.0475 gram sucrose.

158. Modified Violette Method.—The alkaline copper solution with modifications used generally by analysts in this country is based on the solution proposed by Violette.

The sugar solution employed should contain approximately one per cent. of reducing sugar. If it should have a greater content it should be reduced with water to approximately the one named. If it have a less content, it should be evaporated in a vacuum at a low temperature until it reaches the strength mentioned above. A preliminary test will indicate almost the exact quantity of the sugar solution to be added to secure a complete reduction of the copper. This having been determined the whole quantity should be added at once to a second quantity of the boiling copper solution, the test tube held in the open flame of a lamp giving a large circular flame and the contents of the tube kept in brisk ebullition for just two minutes. The lamp is withdrawn and the precipitated suboxid allowed to settle. If a distinct blue color remain an additional quantity of the sugar solution is added and again boiled a few seconds. When the blue coloration is no longer distinct, the presence or absence of copper is determined by filtering a drop or two of the hot solution with the apparatus described below. This clear filtered liquor is then brought into contact with a drop of potassium ferrocyanid solution acidulated with acetic. The production of a brown precipitate or color indicates that some copper is still present, in which case an additional quantity of the sugar solution is added and the operation continued as described above until after the last addition of sugar solution no coloration is produced. Many chemists prefer to remove a drop of the solution and place it on a piece of filter paper that has been treated with the acidified potassium ferrocyanid.

159. The Filtering Tube.—The filtering tube used in the above operation is made of narrow glass tubing not over one-quarter inch inside diameter with thick walls. The length of the tube should be from nine to ten inches. One end of the tube being

softened in the flame is pressed against a block of wood so as to form a flange. Over this flange is tied a piece of fine linen.⁶⁸

Instead of using a linen diaphragm the tube is greatly improved, as suggested by Knorr, by sealing into the end of the tube while hot a perforated platinum disk. Before using, the tube is dipped into a vessel containing some suspended asbestos felt and by aspiration a thin felt of asbestos is formed over the outer surface of the platinum disk. By inverting the tube the liquid which has entered during aspiration is removed. The tube thus prepared is dipped into the boiling solution in the test tube above described and aspiration continued until a drop of the liquor has entered the tube. It is then removed from the boiling

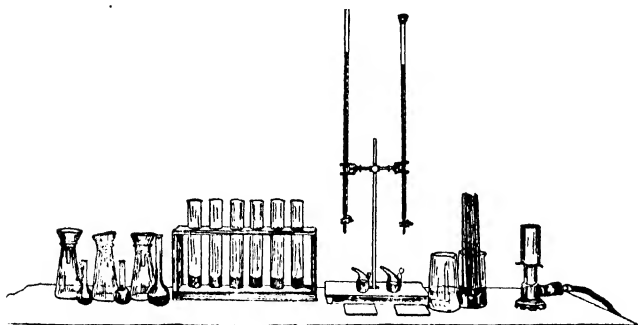


Figure 51.—Apparatus for the Volumetric Estimation of Reducing Sugars.

solution, the asbestos felt wiped off with a clean towel, and the drop of liquor in the tube blown through the openings in the platinum disk and brought into contact with a drop of potassium ferrocyanid in the usual way. In this way a drop of the liquor is secured without any danger of a reoxidation of the copper which may sometimes take place on cooling.

The careful analyst by working in this way with the volumetric method is able to secure highly accurate results. The apparatus used is shown in the accompanying illustration. From

⁶⁸ Wiley, *Bulletin de l'Association des Chimistes de Sucrerie et de Distillerie*, 1884, 2: 108.

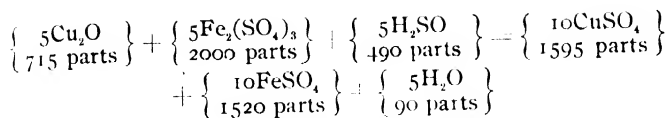
the number of cubic centimeters of sugar solution used to decolorize the solution, the percentage of reducing sugar can be determined. As copper sulfate is liable to contain impurities, it is imperative that the Violette solution as well as any of the other volumetric solutions should be standardized against a known sugar solution and the value of each be determined.

This standardization should be often repeated during the use of the solution so as to note any change which may occur on standing.

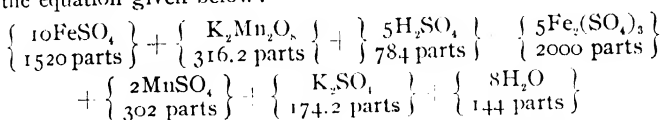
On account of this solution being easily decomposed on standing and its sugar value thereby changing, the following modification is used. The 34.64 grams of copper sulfate are dissolved in water and made up to one liter, and the other ingredients are dissolved in water and likewise made up to one liter. The solution being kept in separate bottles. For a determination, 10 cubic centimeters of each of the reagents are taken instead of 10 cubic centimeters of the original Violette solution and 10 cubic centimeters of water.

The reduction is accomplished in a long test tube at least one and one-half inches in diameter and nine inches long. The mixed solution is heated to the boiling point over the naked flame of a burner, then a few cubic centimeters of the prepared sugar solution are added and boiled two minutes. Repeat this operation until the blue color almost disappears, taking care to add the juice little by little as this point is approached and then only a drop or two at a time until the blue color disappears. After the first boiling of two minutes, it is only necessary to boil the liquid a few seconds, after each addition.

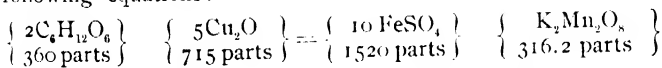
160. Permanganate Process for the Estimation of Reducing Sugars.—The principle of the process is based upon the observation that two molecules of dextrose reduce from an alkaline cupric tartrate solution five molecules of cuprous oxid. The five molecules of cuprous oxid thus precipitated when added to an acid solution of ferric sulfate, will change five molecules of the ferric sulfate to ten molecules of ferrous sulfate. The reaction is illustrated by the following equation:



The 10 molecules of ferrous sulfate formed as indicated in the above reaction, are reoxidized to ferric sulfate by a set solution of potassium permanganate. This reaction is illustrated by the equation given below:



By the study of the above equations it is seen that two molecules of dextrose or other similar reducing sugar, are equivalent to one molecule of potassium permanganate, as is shown by the following equations:



It is thus seen that 316.2 parts by weight of potassium permanganate are equivalent to 360 parts by weight of dextrose; or one part of permanganate corresponds to 1.1385 parts by weight of dextrose. If, therefore, the amount of permanganate required in the above reaction to restore the iron to the ferric condition, be multiplied by the factor mentioned above, the quotient will represent in weight the amount of dextrose which enters into the reaction. The standard solution of potassium permanganate should contain 4.392 grams of the salt in a liter. One cubic centimeter of this solution is equivalent to five milligrams of dextrose.

161. Manipulation.—The saccharine solution whose strength is to be determined should contain approximately about one per cent. of sugar. Of this solution 10 cubic centimeters are placed in a porcelain dish together with a considerable excess of Fehling solution. When no sucrose is present, the mixture may be heated to the temperature of boiling water and kept at that temperature for a few minutes until all the reducing sugar is oxid-

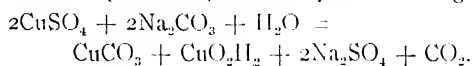
ized. There should be enough of the copper solution used to maintain a strong blue coloration at the end of the reaction. A greater uniformity of results will be secured by using in all cases a considerable excess of the copper solution. When sucrose or other non-reducing sugars are present, the temperature of the reaction should not be allowed to exceed 80 degrees and the heating may be continued somewhat longer. At this temperature the copper solution is without action on sucrose. The precipitated suboxid is allowed to settle, the supernatant liquid poured off through a Gooch crucible and the suboxid washed thoroughly a number of times by decantation with recently boiled hot water, and collected in the crucible where it should be also thoroughly washed. The suboxid is dissolved in a solution of ammonio-ferric sulfate containing an excess of sulfuric acid and made up to half a liter. It is then titrated with the set solution of potassium permanganate described above.

162. Modified Permanganate Method.—The asbestos, with as much of the precipitate as possible, is transferred to the beaker in which the precipitation was made, beaten up with from 25 to 30 cubic centimeters of hot, recently boiled water, and from 50 to 75 cubic centimeters of a saturated solution of ammonio-ferric sulfate in 25 per cent. sulfuric acid are added to the beaker and then poured through the crucible to dissolve the cuprous oxid remaining therein. If the precipitate be first beaten up with water as directed, so that no large lumps of it remain, there is no difficulty in dissolving the oxid in the ferric salt; while if any lumps of the oxid be allowed to remain there is great difficulty. After the solution is obtained, it is titrated with a solution of potassium permanganate of such a strength that each cubic centimeter is equivalent to 0.01 gram of copper.

The permanganate solution used should be standardized by means of metallic iron, but in ordinary work it is also recommended to standardize by check determinations of reducing sugars in the same sample by the gravimetric method.

The method seems to be sufficiently accurate for all ordinary purposes and is extremely rapid.

163. Copper Carbonate Process (Soldaini's Solution).—While the copper solutions which have been mentioned in previous paragraphs have only a slight action on sucrose and dextrin yet on prolonged boiling even these bodies show a reducing effect due probably to a preliminary change in the sugar molecules whereby products analogous to dex'trose or invert sugar are formed. In order to secure a reagent, which was little affected by the sucrose and non-reducing sugars, Soldaini has proposed to employ a liquor containing the copper as carbonate instead of as tartrate.⁶⁹ This solution is prepared by adding to a solution of 40 grams of copper sulfate one of equal strength of sodium carbonate. The resulting copper carbonate and hydroxid are collected on a filter, washed with cold water, and dried. The reaction which takes place is represented by the following formula:



The dry precipitate obtained, which will weigh about 15 grams, is placed in a large flask with about 416 grams of potassium bicarbonate and 1,400 cubic centimeters of water. The contents of the flask are heated on a steam-bath for several hours with occasional stirring until the evolution of carbon dioxide has ceased. During this time the liquid is kept at the same volume by the addition of water, or by attaching a reflux condenser to the flask. The two residual copper compounds at the end of this time will be found dissolved and the resulting liquor will have a deep blue color. After filtration the solution is boiled for a few minutes and cooled to room temperature. The volume is then completed to two liters. The specific gravity of the solution should be approximately 1.185. A more direct method of preparing the solution, and one quite as effective, consists in adding the solution of the copper sulfate directly to the hot solution of potassium bicarbonate and heating and shaking the mixture until the copper carbonate formed is dissolved. After filtering the volume is made as above. The proportions of reagents employed are placed by Preuss at 15.8 grams of crys-

⁶⁹ *Gazetta Chimica Italiana* 1876, 6:322.

tallized copper sulfate and 594 grams of potassium bicarbonate.⁷⁰ The soldaini reagent is extremely sensitive and is capable of detecting as little as half a milligram of invert sugar. The presence of sucrose makes the reagent more delicate, and it is especially useful in determining the invert sugar arising during the progress of manufacture by the action of heat and mclassigenic bodies on sucrose.

164. The Analytical Process.—As in the case of Fehling solution a great many methods of conducting the analysis with the Soldaini reagent have been proposed. The general principle of all these processes is the one already described for the alkaline copper tartrate solution, *viz.*, the addition of the reducing sugar solution to the boiling reagent, and the determination of the end of the reaction by the disappearance of the copper.⁷¹

In this case, however, these methods have had no general application, and the use of the Soldaini reagent has been confined chiefly to the determination of invert sugar in presence of a large excess of sucrose. For this purpose the sugar solution is not added until the blue color of the reagent has been destroyed, but on the other hand, the reagent has been used in excess, and the cuprous oxid formed collected and weighed as metallic copper. The weight of the metallic copper found, multiplied by the factor 0.3546, gives the weight of invert sugar in the volume of the sugar solution used. According to Preuss, the factor is not a constant one, but varies with the quantity of invert sugar present, as is seen in the formula $y = -8.368537 + 0.55346x + 0.00003216x^2$, in which y = the invert sugar, and x the metallic copper.⁷²

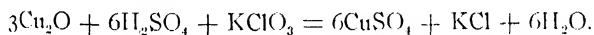
165. Sidersky's Modification of Soldaini's Process.—In all cases where the sugar solutions are not too highly colored, Sidersky finds that the method of reduction in a large test tube, as practised by Violette, is applicable with the copper carbonate solu-

⁷⁰ Sidersky, *Traité d'Analyse des Natières Sucrées*, 1890: 148.

⁷¹ Sidersky, *Traité d'Analyse des Matières Sucrées*, 1890: 149.

⁷² *Zeitschrift des Vereins für Rübenzucker-Industrie* 1888, **38**, Neuer folge **25**: 727.

tion.⁷³ For more exact work or where the solutions are dark in color it is preferred to determine the quantity of copper reduced by an indirect volumetric method. The sugar solution, properly clarified and the lead removed by sodium carbonate, dry or in solution, is made of such a volume as to contain less than one per cent. of reducing sugars. In a flask or large test tube are placed 100 cubic centimeters of the Soldaini copper solution, which is boiled for a short time and the sugar solution added, little by little, from a pipette, at such a rate as not to stop the ebullition. The boiling is continued for five minutes after the last addition of the sugar. The vessel is taken from the flame and 100 cubic centimeters of cold water added, the whole brought on an asbestos felt preferably in a Gooch crucible and the cuprous oxid washed with hot water until the alkaline reaction has disappeared. Three or four washings are generally necessary. The residual cuprous oxid is dissolved in a measured quantity of normal, semi-normal or fifth normal sulfuric acid. In case of normal, 25 cubic centimeters are generally sufficient; a few particles of potassium chlorate added, and the mixture boiled until the cuprous oxide is in solution. The reaction is represented by the following formula:



The residual sulfuric acid is titrated with a half-normal alkali ammonia being preferred.

The solution of ammonia is made by diluting 200 cubic centimeters of commercial aqua ammonia with 800 of water. Its strength is determined by titrating 25 cubic centimeters, to which has been added two cubic centimeters of a concentrated copper sulfate solution as indicator with the normal solution of sulfuric acid until the blue color disappears, adding sufficient water to the ammonia solution to make it half normal. The copper sulfate secured from the cuprous sulfate as described above is cooled, and a quantity of the ammonia, equal to 25 cubic centimeters of the set sulfuric acid, added. The excess of the am-

⁷³ Sidersky, *Bulletin de l'Association des Chimistes*, 1886, **4**:177; and 1888, **6**:233.

monia is then determined by titration with the sulfuric acid, the disappearance of the blue color being the indication of the end of the reaction. The number of cubic centimeters of the set sulfuric acid required to saturate the ammonia represents the equivalent of cuprous oxid originally present. One cubic centimeter of normal sulfuric acid is equivalent to 0.0317 gram of metallic copper.

To determine the weight of invert sugar oxidized, multiply the weight of copper, calculated as above described, by the factor 0.3546.⁷⁴ For a general application of this method of analysis the relative quantities of copper reduced by different quantities of sugar must be taken into consideration.

While, as has already been stated, the copper carbonate process has heretofore been applied chiefly to the detection of invert sugar, it has merits which justify the expectation that it may some time supplant the Fehling liquor both for volumetric and gravimetric work. Large volumes of the reagent can be prepared at once and without danger of subsequent change. The action of the reagent on the hexobioses and trioses is far less vigorous than that of the alkaline copper tartrate, and the end reactions for volumetric work are, at least, as easily determined in the one case as the other.

166. Relation of Reducing Sugar to Quantity of Copper Suboxid Obtained.—The relation of the quantity of copper reduced to the amount of sugar oxidized by the copper carbonate solution has been determined by Ost, and the utility of the process thereby increased.⁷⁵ The solution used should have the following composition: 23.5 grams of crystallized copper sulfate, 250 grams of potassium bicarbonate and 100 grams of sodium bicarbonate in one liter. Without an indicator the end reaction is distinctly marked by the passage of the blue color into a colorless solution. Ost affirms that this solution is preferable to any form of Fehling liquor because it can be kept indefinitely unchanged; it attacks

⁷⁴ Bodenbender and Sheller, *Zeitschrift des Vereins für die Rübenzucker-Industrie*, 1887, **37**, Neuer folge 24 : 138.

⁷⁵ *Berichte der deutschen Chemischen Gesellschaft*, 1890, **23**:3003. *Zeitschrift des Vereins für die Rübenzucker-Industrie*, 1891, **41**, Neuer folge 28 : 97.

sucrose far less strongly, and an equal quantity of sugar precipitates nearly double the quantity of copper. The boiling requires a longer time, as a rule 10 minutes, but this is a matter of no importance, when the other advantages are taken into consideration. The relations of the different sugars to the quantity of copper precipitated are given in the table on page 196.

167. Factor for Different Sugars.—For pure dextrose the relation between sugar and copper reduced has been determined by Ost. The data were obtained by adding to 50 cubic centimeters of the copper solution 25 cubic centimeters of sugar solutions of varying strength and collecting, washing, and reducing the cuprous oxid obtained in a current of hydrogen in a glass tube by the method described further on.

The boiling in all cases was continued just 10 minutes, although a slight variation from the standard time did not produce so great a difference as with Fehling reagent. In the case of dextrose, when 50 milligrams were used with 50 cubic centimeters of the solution, the milligrams of copper obtained after six, 10 and 20 minutes' boiling were 164.6, 165.5, and 166.9 respectively.⁷⁶

The data differ considerably from those obtained by Herzfeld, but in his experiments the boiling was continued only for five minutes, and this is not long enough to secure the proper reduction of the copper.⁷⁷

AMMONIACAL COPPER SOLUTION.

168. Pavy's Process.—The well-known solubility of cuprous oxid in ammonia led Pavy to adopt a copper reagent containing ammonia in the volumetric determination of reducing sugars.⁷⁸ In Pavy's process an alkaline copper solution is employed made up in the usual way, to which a sufficient quantity of ammonia is added to hold in solution all the copper when precipitated as

⁷⁶ *Zeitschrift des Vereins für die Rübenzucker-Industrie*, 1891, **41**, Neuer folge 28: 97.

⁷⁷ *Zeitschrift des Vereins für die Rübenzucker-Industrie*, 1890, **40**, Neuer folge 27: 187.

⁷⁸ *Chemical News* 1879, **39**: 77.

TABLE SHOWING THE QUANTITY OF COPPER REDUCED BY DIFFERENT SUGARS IN THE COPPER CARBONATE METHOD.

Copper Milligrams	Invert Sugar Milligrams	Dextrose Milligrams	Levinose Milligrams	Galactose Milligrams	Arabinose Milligrams
50	15.2	15.6	14.7	17.4	17.0
55	16.6	17.0	16.1	19.1	18.6
60	18.0	18.5	17.5	20.8	20.3
65	19.4	19.9	18.9	22.5	21.9
70	20.8	21.4	20.3	24.2	23.5
75	22.3	22.9	21.7	25.9	25.1
80	23.7	24.1	23.0	27.7	26.7
85	25.2	25.8	24.3	29.3	28.3
90	26.6	27.3	25.7	31.1	29.9
95	28.1	28.8	27.1	32.8	31.5
100	29.5	30.3	28.5	34.5	33.1
105	31.0	31.8	29.9	36.2	34.7
110	32.4	33.3	31.2	38.0	36.3
115	33.9	34.8	32.6	39.7	37.9
120	35.3	36.3	34.0	41.4	39.5
125	36.8	37.8	35.4	43.1	41.1
130	38.2	39.3	36.8	44.8	42.8
135	39.7	40.8	38.2	46.5	44.4
140	41.1	42.3	39.6	48.3	46.0
145	42.6	43.8	41.0	50.0	47.6
150	44.0	45.3	42.5	51.8	49.3
155	45.5	46.8	43.9	53.6	50.9
160	47.0	48.3	45.3	55.4	52.6
165	48.5	49.8	46.7	57.2	54.3
170	50.0	51.4	48.1	59.0	55.9
175	51.5	52.9	49.5	60.8	57.5
180	53.0	54.5	51.0	62.7	59.2
185	54.5	56.0	52.5	64.5	60.9
190	56.0	57.6	54.0	66.4	62.7
195	57.5	59.2	55.5	68.3	64.4
200	59.1	60.8	57.0	70.3	66.2
205	60.7	62.4	58.6	72.3	68.0
210	62.4	64.1	60.2	74.3	69.8
215	64.1	65.8	61.8	76.3	71.6
220	65.8	67.5	63.5	78.3	73.5
225	67.5	69.2	65.2	80.3	75.4
230	69.3	70.9	66.9	82.4	77.3
235	71.1	72.7	68.7	84.5	79.3
240	72.9	74.5	70.6	86.6	81.3
245	74.8	76.4	72.5	88.9	83.4
250	76.7	78.4	74.4	91.2	85.5
255	78.6	80.5	76.5	93.5	87.6
260	80.5	82.8	78.8	95.9	89.8
265	82.5	85.1	81.1	98.3	92.2
270	84.7	87.5	83.5	100.7	94.6
275	87.1	89.9	85.9	103.3	97.1
280	89.7	92.4	88.6	106.1	99.6
285	92.3	94.9	91.3	109.0	102.3
290	95.1	97.6	94.2	112.0	105.1
295	98.0	100.4	97.2	115.1	107.9
298	100.0	102.5	99.0	117.0	109.5

cuprous oxid. The solution used by Pavy has the following composition: One liter contains

Crystallized copper sulfate	34.65 grams
Potassium-sodium tartrate.....	173.00 "
Caustic potash.....	160.00 "

For use 120 cubic centimeters of the above reagent are mixed with 300 of ammonia of specific gravity 0.88, and the volume completed to one liter with distilled water. Twenty cubic centimeters of this reagent are equivalent to 10 milligrams of dextrose or invert sugar when added in a one per cent. solution.

In the use of ammoniacal copper solution, care must be taken that all the liquids employed be entirely free of oxygen and that the contents of the flask in which the reduction takes place be in some way excluded from contact with the air. Pavy secured this by conducting the reduction in a flask closed with a stopper carrying two holes; one of these served for the introduction of the burette carrying the sugar solution and the other carried a tube dipping into a water seal by means of a slit rubber tube, which would permit of the exit of the vapors of steam and ammonia, but prevent the regurgitation of the water into the flask.

The complete decoloration of the copper solution marks the end of the reaction. The usual precautions in regard to the length of the time of boiling must be observed.

It is easy to see that in the Pavy process the quantity of ammonia in the solution is rapidly diminished during the boiling and this has led to the suggestion of other methods to exclude the air. Among these have been recommended the introduction of a current of hydrogen or carbon dioxid. One of the best methods of procedure is that proposed by Allen, who recommends covering the copper solution by a layer of paraffin oil (Kerosene).⁷⁹

169. Process of Peska.—Peska has also independently made use of Allen's method of covering the solutions with a layer of paraffin oil and finds it reliable.⁸⁰ The copper reagent employed by him has the following composition:

⁷⁹ *The Analyst*, 1894, **19**: 181.

⁸⁰ *Chemical News*, 1895, **71**: 235.

Crystallized copper sulfate.....	6.927 grams
Ammonia, twenty-five per cent strength.....	160.00 cc.

The copper sulfate is dissolved in water, the ammonia added, and the volume completed to half a liter with distilled water. A second solution containing half a liter is made by dissolving 34.5 grams of potassium-sodium tartrate and 10 grams of sodium hydroxide and completing the cool solution to half a liter with distilled water. In all cases the water used in making up the above solutions must be freshly boiled to exclude the air.

For titration, 50 cubic centimeters of each of the above solutions are taken, mixed and covered with a layer of paraffin oil half a centimeter in depth. The reduction is not accomplished at a boiling temperature, but at from 80° to 85°. The manipulation is conducted as follows:

The mixed solutions are placed in a beaker, covered with oil, and heated to 80 degrees. The temperature is measured by a thermometer which also serves as a stirring rod. The sugar solution is run down the sides of the beaker from a burette so adjusted as to be protected from the heat. After each addition of the sugar solution the mixture is carefully stirred, keeping the temperature at from 80 degrees to 85 degrees. The first titration is made to determine approximately the quantity of sugar solution necessary to decolorize the copper. This done, the actual titration is accomplished by adding at once the total amount of sugar solution necessary to decolorize, less about one cubic centimeter. Any sugar solution adhering to the side of the beaker is washed down by distilled water, the contents of the beaker well stirred, and the temperature kept at 85 degrees for two minutes. The rest of the sugar solution is then added in quantities of one-tenth of a cubic centimeter until the decoloration is completed. The total time of the final titration should not exceed five minutes. The sugar solution should be as nearly as possible of one per cent. strength. If a lower degree of strength be employed a larger quantity of the sugar is necessary to reduce a given quantity of copper.

In the case of dextrose, when a one per cent. solution is used, eight and two-tenths cubic centimeters, corresponding to 80.2

milligrams of dextrose, are required to reduce 100 cubic centimeters of the mixed reagent. On the other hand, when the sugar solution is diluted to one-tenth of a per cent. strength 82.1 milligrams are required.

With invert sugar slightly larger quantities are necessary, the reducing power being as 94.9 to 100 as compared with dextrose. With a one per cent. strength of invert sugar it is found that 84 milligrams are required to reduce 100 cubic centimeters of the mixed reagent and when the strength of the invert sugar is reduced to one-tenth per cent. 87.03 milligrams are required.

170. Method of Allein and Gaud.—Allein and Gaud have proposed a further modification of the ammonia process which consists essentially in the suppression of rochelle salt and fixed caustic alkali and the entire substitution therefor with ammonia. Ammonia acts with much less vigor upon sugars than the caustic alkalies, and it is therefore claimed that the decomposition of the sugar due to the alkali is reduced to a minimum when ammonia is employed.⁸¹ The copper solution is made as follows:

Dissolve 8.7916 grams of electrolytic copper in 93 grams of concentrated sulfuric acid diluted with an equal volume of water. Complete the resulting solution to one liter with concentrated ammonia. Ten cubic centimeters of this solution are equal to 50 grams of dextrose.

It is recommended that the reduction be accomplished in an atmosphere of hydrogen, but it is apparent that the use of kerosene is permissible in this case, and on account of its greater simplicity it is to be recommended as the best means of excluding the oxygen. The reduction is accomplished at a temperature of about 80 degrees.

It is also proposed to reoxidize the copper by substituting a current of air for the hydrogen at the end of the reaction, and thus use the same copper a number of times. The danger of loss of ammonia, and the difficulty of determining when the oxidation is complete, render this regeneration of the reagent undesirable.

⁸¹ *Journal de Pharmacie et de Chimie*, 1894, 30: 305.

171. Method of Gerrard.—The method of Gerrard does not depend upon the use of ammonia, but the principle involved is the same, viz., the holding of the separated cuprous oxid in solution and the determination of the end of the reaction by the disappearance of the blue color. As first proposed by Gerard, the copper sulfate solution is made of double the strength usually employed and to each 100 cubic centimeters thereof, before use, three and three-tenths grams of potassium cyanid are added. This is sufficient to hold the precipitated cuprous oxid in solution.⁸²

The original method of Gerrard is found difficult of execution and the author, in conjunction with Allein, has lately modified it and reduced it to a practical working basis.⁸³

In the new method the ordinary Fehling solution is employed and it is prepared for use in the following way: Ten cubic centimeters of the Fehling solution, or half that quantity of each of the component parts kept in separate bottles, are placed in a porcelain dish with 40 cubic centimeters of water and brought to the boiling-point. To the boiling liquid is added, from a pipette, a five per cent. solution of potassium cyanid until the blue color just disappears, or only a very faint tint of blue remains, avoiding any excess of the cyanid. A second portion of the Fehling solution equal to that first employed is added, and to the boiling mixture the solution of sugar is added, from a burette, until the blue color disappears. The contents of the dish should be kept boiling during the addition of the sugar solution. The volume used will contain 50 milligrams of dextrose. The sugar solution should be of such a strength as to contain no more than half a per cent. of reducing sugar.

The principle of the preparation of the solution may be stated as follows: If to a solution of copper sulfate, potassium cyanid be added until the blue color disappears, a double cyanid of copper and potassium is formed according to the following reaction: $\text{CuSO}_4 + 4\text{KCN} = \text{Cu}(\text{CN})_2 \cdot 2\text{KCN} + \text{K}_2\text{SO}_4$. This double

⁸² *Pharmaceutical Journal* [3], 1892-93, 23 : 208.

⁸³ *Pharmaceutical Journal* [3] 1894-95, 25 : 913.

cyanid is a salt of considerable stability. It is not decomposed by alkalies, hydrogen or ammonium sulfid. With mineral acids it gives a whitish curdy precipitate. With Fehling solution the same double cyanid is formed as that described above. If, however, Fehling solution be present in excess of the amount necessary to form the double cyanid of copper, this excess can be used in the oxidation of reducing sugar and the colorless condition of the solution will be restored as soon as the excess of the Fehling is destroyed. The double cyanid holds in solution the cuprous oxid formed and thus complete decoloration is secured.

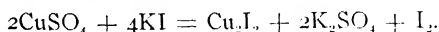
172. Method Depending on Titration of Excess of Copper.— Instead of measuring the quantity of copper reduced, either by its disappearance or by reducing the cuprous oxid to a metallic state, Politis has proposed a method of analysis depending on the titration of the residual copper.⁴¹ The reagents employed are:

(1) A copper solution containing 24.95 grams of crystallized copper sulfate, 140 grams of sodium and potassium tartrate, and 25 grams of sodium hydroxid in one liter:

(2) A solution of sodium thiosulfate containing 24.8 grams of the salt in one liter:

(3) A solution of potassium iodid containing 12.7 grams of iodin in one liter.

The reaction is represented by the formula



The analytical process is carried out as follows: In a 100 cubic centimeter flask are boiled 50 cubic centimeters of the copper solution, 10 cubic centimeters of about one-tenth per cent. reducing sugar solution are added, the boiling continued for five minutes, the flask filled to the mark with boiling water and its contents filtered. Fifty cubic centimeters of the hot filtrate are cooled, slightly acidified, potassium iodid solution added in slight excess, and the iodine set free determined by titration with sodium thiosulfate. The quantity of iodine obtained corresponds

⁴¹ Politis, *Zeitschrift des Vereins für die Rübenzucker-Industrie*, 1889, 39, *Neur folge* 26: 935.

to the unreduced copper remaining after treatment with the reducing sugar. The number of cubic centimeters of thiosulfate used subtracted from 25 will give the number of cubic centimeters of the copper solution which would be reduced by five cubic centimeters of the sugar solution used.

Example.—In the proportions given above it was found that 11 cubic centimeters of thiosulfate were required to saturate the iodine set free. Then $25 - 11 = 14$ cubic centimeters of copper solution reduced by five cubic centimeters of the sugar solution. Since one cubic centimeter of the copper solution is reduced by 0.0036 gram of dextrose the total dextrose in the five cubic centimeters $= 0.0036 \times 5 = 0.0180$ gram.

The above method does not seem to have any practical advantage over those based on noting the disappearance of the copper and is given only to illustrate the principle of the process. While the titration of the iodine by sodium thiosulfate is easily accomplished in the absence of organic matter, it becomes difficult, as shown by Ewell, when organic matters are present, as they always are in the oxidation of a sugar solution. Ewell has therefore proposed to determine the residual copper by a standard solution of potassium cyanide, but the method has not yet been developed.⁸⁵

GRAVIMETRIC REDUCING SUGAR METHODS.

173. General Principles.—In the preceding pages the principles of the volumetric methods of reducing sugar analysis by means of alkaline copper solution have been set forth together with the exact procedure to be followed in each method. They depend either on the total decomposition of the copper solution employed by the reducing sugar, or else on the collection and titration of the cuprous oxide formed in the reaction. In the gravimetric methods to be discussed the general principle of the process rests upon the collection of the cuprous oxide formed the weighing as such or as cupric oxide or its reduction to metallic copper, the weight of which serves as a starting point in the calculations of

⁸⁵ Ewell, Manuscript communications to author.

the weight of reducing sugar which has been oxidized in the reaction.

174. Electrolytic Copper Reduction Method.—The reduction of the copper solution and the electrolytic deposition of the copper are accomplished as follows:

The copper and alkali solutions are kept in separate bottles. After mixing the equivalent volume of the two solutions in a beaker, heat is applied and the mixture boiled. To the boiling liquid the proper volume of the cold sugar solution is added. This must always be less than the amount required for complete reduction. The solution is again brought into ebullition and kept boiling exactly two minutes. A two-minute sand glass is conveniently used to determine the time of boiling. At the end of this time an equal volume of freshly boiled cold water is added, and the supernatant liquor at once passed through a gooch under vacuum. The residual cuprous oxid is covered with boiling water and washed by decantation until the wash water is no longer alkaline. It is more convenient to wash in such a way that, at the end, the greater part of the cuprous oxid is in the gooch. The felt and cuprous oxid are then returned to the beaker in which the reduction is made. The gooch is moistened with nitric acid to dissolve any adhering oxid and then is washed into the beaker. Enough nitric acid is added to bring all the oxid into solution, an excess being avoided, and a small amount of water added. The mixture is again passed by suction through a gooch having a thin felt, to remove the asbestos and the filtrate collected in a flask of about 150 cubic centimeters capacity. The washing is continued until the gooch is free of copper, when the volume of the filtrate should be about 100 cubic centimeters. The liquid is transferred to a platinum dish holding about 175 cubic centimeters and the flask washed with about 25 cubic centimeters of water. From three to five cubic centimeters of strong sulfuric acid are added and the copper deposited by an electric current.

175. Precipitating the Copper.—When no more nitric acid is

used than indicated in the previous paragraph, it will not be necessary to remove it by evaporation. The platinum dishes containing the solutions of the cuprous oxid are arranged as shown in the figure for the precipitation of the copper by the electric current. Each of the supporting stands has its base covered with sheet-copper, on which the platinum dishes rest. The uprights are made of heavy glass rods and carry the supports for the platinum cylinders which dip into the copper solutions. The current used is from the city service and is brought in through the lamp shown at the right of the figure. This current has a voltage of about 120. After passing the lamp it is conducted through the regulator shown at the right, a glass tube closed below by a stopper carrying a piece of platinum foil, and above by one holding a glass tube, in the lower end of which is sealed a piece of sheet platinum connected, through the glass tube, with the lamp. The regulating tube contains dilute sulfuric acid. The strength of current desired is secured by adjusting the movable pole. A battery of this kind easily secures the precipitation of sixteen samples at once, but only twelve are shown in the figure. The best practice is to start the operation at the time of leaving the laboratory in the afternoon. The next morning the deposition of the copper will be found complete. The wiring of the apparatus is shown in the figure. The wire from the regulator is connected with the base of the first stand, and thence passes through the horizontal support to the base of the second, and so on. The return to the lamp is accomplished by means of the upper wire. This plan of arranging the apparatus has been used successfully for a number of years.

Where a street current is not available, the following directions may be followed: Use four gravity cells, such as are employed in telegraphic work, for generating the current. This will be strong enough for one sample and by working longer for two. Connect the platinum dish with the zinc pole of the battery. The current is allowed to pass until all the copper is deposited. Where a large number of samples is to be treated at once, the size of the battery must be correspondingly increased.

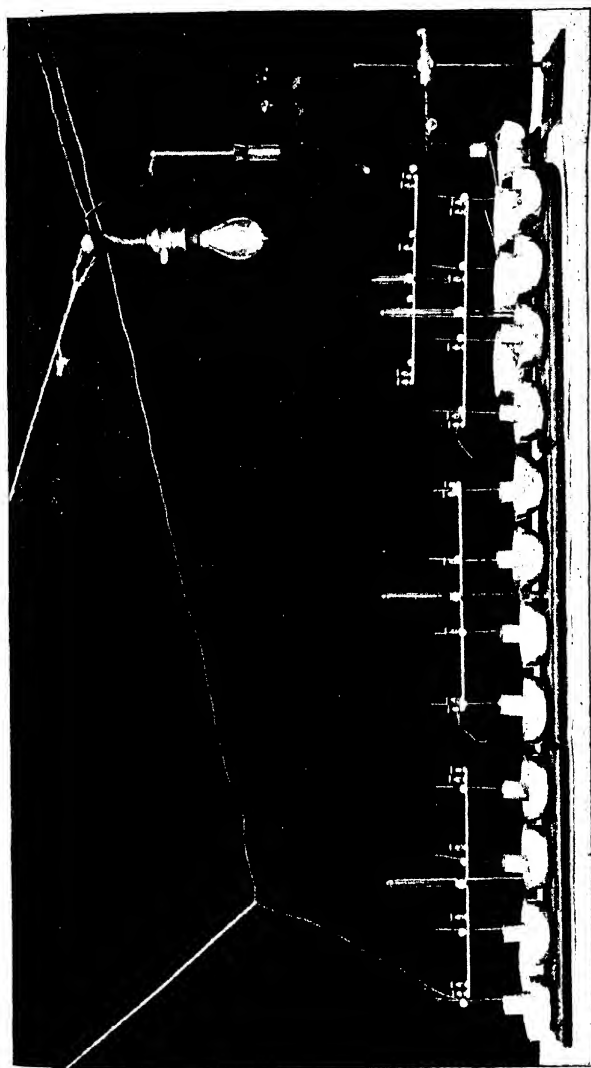


Figure 52.—Apparatus for the Electrolytic Deposition of Copper.

176. Official Methods of Dissolving the Cuprous Oxids.⁸⁶—

(A) *from a sulfuric acid solution.* Filter the cuprous oxid in a gooch, wash the beaker and precipitate thoroughly with hot water without any effort to transfer the precipitate to the filter. Wash the asbestos film and the adhering cuprous oxid into the beaker by means of hot dilute nitric acid. After the copper is all in solution, refilter through a gooch with a thin film of asbestos and wash thoroughly with hot water. Add 10 cubic centimeters of dilute sulfuric acid, containing 200 cubic centimeters of sulfuric acid (specific gravity 1.84) per liter, and evaporate the filtrate on the steam bath until the copper salt has largely crystallized. Heat carefully on a hot plate or over a piece of asbestos board until the evolution of white fumes shows that the excess of nitric acid is removed. Add from eight to 10 drops of nitric acid (specific gravity, 1.42) and rinse into a platinum dish of from 100 to 125 cubic centimeters capacity. Precipitate the copper by electrolysis. Wash thoroughly with water before breaking the current, remove the dish from the circuit, wash with alcohol and ether successively, dry at about 50 degrees and weigh. If preferred, the electrolysis can be conducted in a beaker, the copper being deposited upon a weighed platinum cylinder.

(B) *From sulfuric and nitric acid solution.* Filter and wash as under (A). Transfer the asbestos film from the crucible to the beaker by means of a glass rod and rinse the crucible with about 30 cubic centimeters of a boiling mixture of dilute sulfuric and nitric acids, containing 65 cubic centimeters of sulfuric acid (specific gravity, 1.84) and 50 cubic centimeters of nitric acid (specific gravity, 1.42) per liter. Heat and agitate until solution is complete; filter and electrolyze as under (A).

(C) *From nitric acid solution.* Filter and wash as under (A). Transfer the asbestos film and adhering oxid to the beaker. Dissolve the oxid still remaining in the crucible by means of two cubic centimeters of nitric acid (specific gravity, 1.42), adding it with a pipette and receiving the solution in the beaker containing the asbestos film. Rinse the crucible with a jet of water.

⁸⁶ Bulletin 107, Bureau of Chemistry, 1910: 52.

allow the rinsings to flow into the beaker. Heat the contents of the beaker until the copper is all in solution, filter, dilute the filtrate to a volume of 100 cubic centimeters or more, and electrolyze. When a nitrate solution is electrolyzed, the first washing of the deposit should be made with water acidulated with sulfuric acid, in order that the nitric acid may be all removed before the current is interrupted.

177. Method Used at the Halle Station.—The method used at the Halle station is the same as that originally described by Maercker for dextrose.⁸⁷ The copper solution employed is the

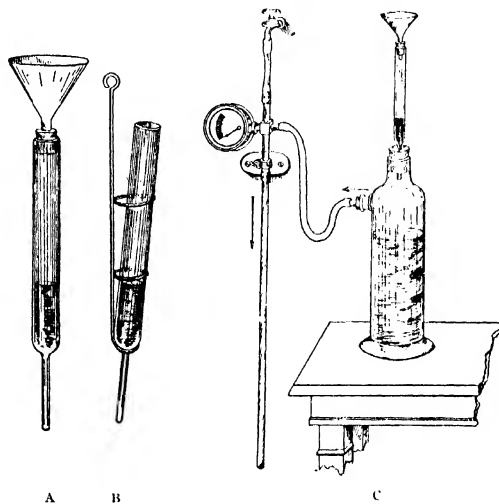


Figure 53. -Apparatus for Filtering Copper Suboxid.

same as in the Allihn method, *viz.*, 34.64 grams of copper sulfate in 500 cubic centimeters, and 173 grams of rochelle salt and 125 grams of potassium hydroxid in the same quantity of water. In a porcelain dish are placed 30 cubic centimeters of copper solution and an equal quantity of the alkali, 60 cubic centimeters of water added and the mixture boiled. To the solution, in lively ebullition, are added 25 cubic centimeters of the

⁸⁷ Maercker, *Handbuch der Spiritusfabrication*, 9 aufl., 1908: 146.

dextrose solution to be examined which must not contain more than one per cent. of sugar. The mixture is again boiled and the separated cuprous oxid immediately poured into the filter and washed with hot water, until the disappearance of an alkaline reaction. For filtering, a glass tube is employed, provided with a platinum disk, and resembling in every respect similar tubes used for the extraction of substances with ether and alcohol. The arrangement of the filtering apparatus for suction is shown in C, Fig. 53. B, Fig. 53, shows a wire holder for the tube. It is recommended that the tubes be prepared by introducing a platinum cone in place of the platinum disk and filling it with

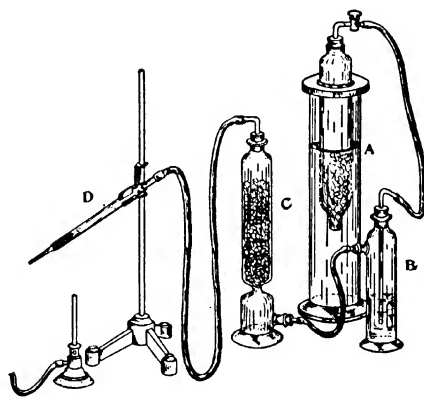


Figure 54. Apparatus for Reducing Copper Suboxid.

asbestos felt, pressing the felt tightly against the sides of the glass tube and making the asbestos fully one centimeter in thickness. This is a much less convenient method of working than the one described above. After filtration and washing, the cuprous oxid is washed with ether and alcohol and dried for an hour at 110 degrees, and finally reduced to metallic copper in a stream of pure dry hydrogen, heat being applied by means of a small flame. The apparatus for the reduction of the cuprous oxid is shown in Fig. 54. In this cut the tube is shown at D.

A is an apparatus for generating hydrogen, B a wash bottle and C a calcium chlorid tube for drying. The metallic copper, after cooling and weighing, is dissolved in nitric acid, the tube washed with water, ether and alcohol, and again dried, when it is ready for use a second time. The percentage of dextrose is calculated from the milligrams of copper found by Allihn's table.

178. Tables for Use in the Gravimetric Determination of Reducing Sugars.—The value of a table for computing the percentage of a reducing sugar present in a solution, is based on the accuracy with which the directions for the determination are followed. The solution must be of the proper strength and made in the way directed. The degree of dilution prescribed must be scrupulously preserved and the methods of boiling during reduction and washing the reduced copper, followed. The quantity of copper obtained by the use of different alkaline copper solutions and of sugar solutions of a strength different from that allowed by the fixed limits, is not a safe factor for computation. It must be understood, therefore, that in the use of the tables the directions which are given are to be followed in every particular.

179. Allihn's Gravimetric Method for the Determination of Dextrose.—*Reagents:*

I. 34.639 grams of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, dissolved in water and diluted to half a liter:

II. 173 grams of rochelle salts } dissolved in water and diluted
125 grams of KOH, }
to half a liter.

Manipulation: Place 30 cubic centimeters of the copper solution (I), 30 cubic centimeters of the alkaline tartrate solution (II), and 60 cubic centimeters of water in a beaker and heat to boiling. Add 25 cubic centimeters of the solution of the material to be examined, which must be so prepared as not to contain more than one per cent. of dextrose, and boil for two minutes. Filter immediately after adding an equal volume of

recently boiled cold water and obtain the weight of copper by one of the gravimetric methods given. The corresponding weight of dextrose is found by the following table:

ALLIHN'S TABLE FOR THE DETERMINATION OF DEXTROSE.

Milli-grams of cop- per	Milli-grams of dex- trose	Milli-grams of cop- per	Milli-grams of dex- trose	Milli-grams of cop- per	Milli-grams of dex- trose	Milli-grams of cop- per	Milli-grams of dex- trose	Milli-grams of cop- per	Milli-grams of dex- trose
10	6.1	46	23.9	82	41.8	118	60.1	154	78.6
11	6.6	47	24.4	83	42.3	119	60.6	155	79.1
12	7.1	48	24.9	84	42.8	120	61.1	156	79.6
13	7.6	49	25.4	85	43.4	121	61.6	157	80.1
14	8.1	50	25.9	86	43.9	122	62.1	158	80.7
15	8.6	51	26.4	87	44.4	123	62.6	159	81.2
16	9.0	52	26.9	88	44.9	124	63.1	160	81.7
17	9.5	53	27.4	89	45.4	125	63.7	161	82.2
18	10.0	54	27.9	90	45.9	126	64.2	162	82.7
19	10.5	55	28.4	91	46.4	127	64.7	163	83.3
20	11.0	56	28.8	92	46.9	128	65.2	164	83.8
21	11.5	57	29.3	93	47.4	129	65.7	165	84.3
22	12.0	58	29.8	94	47.9	130	66.2	166	84.8
23	12.5	59	30.3	95	48.4	131	66.7	167	85.3
24	13.0	60	30.8	96	48.9	132	67.2	168	85.9
25	13.5	61	31.3	97	49.4	133	67.7	169	86.4
26	14.0	62	31.8	98	49.9	134	68.2	170	86.9
27	14.5	63	32.3	99	50.4	135	68.8	171	87.4
28	15.0	64	32.8	100	50.9	136	69.3	172	87.9
29	15.5	65	33.3	101	51.4	137	69.8	173	88.5
30	16.0	66	33.8	102	51.9	138	70.3	174	89.0
31	16.5	67	34.3	103	52.4	139	70.8	175	89.5
32	17.0	68	34.8	104	52.9	140	71.3	176	90.0
33	17.5	69	35.3	105	53.5	141	71.8	177	90.5
34	18.0	70	35.8	106	54.0	142	72.3	178	91.1
35	18.5	71	36.3	107	54.5	143	72.9	179	91.6
36	18.9	72	36.8	108	55.0	144	73.4	180	92.1
37	19.4	73	37.3	109	55.5	145	73.9	181	92.6
38	19.9	74	37.8	110	56.0	146	74.4	182	93.1
39	20.4	75	38.3	111	56.5	147	74.9	183	93.7
40	20.9	76	38.8	112	57.0	148	75.5	184	94.2
41	21.4	77	39.3	113	57.5	149	76.0	185	94.7
42	21.9	78	39.8	114	58.0	150	76.5	186	95.2
43	22.4	79	40.3	115	58.6	151	77.0	187	95.7
44	22.9	80	40.8	116	59.1	152	77.5	188	96.3
45	23.4	81	41.3	117	59.6	153	78.1	189	96.8

Milli-grams of cop-per	Milli-grams of dex-trose	Milli-grams of cop-per	Milli-grams of dex-trose	Milli-grams of cop-per	Milli-grams of dex-trose	Milli-grams of cop-per	Milli-grams of dex-trose	Milli-grams of cop-per	Milli-grams of dex-trose
190	97.3	233	120.1	276	143.3	319	167.0	362	191.1
191	97.8	234	120.7	277	143.9	320	167.5	363	191.7
192	98.4	235	121.2	278	144.4	321	168.1	364	192.3
193	98.9	236	121.7	279	145.0	322	168.6	365	192.9
194	99.4	237	122.3	280	145.5	323	169.2	366	193.4
195	100.0	238	122.8	281	146.1	324	169.7	367	194.0
196	100.5	239	123.4	282	146.6	325	170.3	368	194.6
197	101.0	240	123.9	283	147.2	326	170.9	369	195.1
198	101.5	241	124.4	284	147.7	327	171.4	370	195.7
199	102.0	242	125.0	285	148.3	328	172.0	371	196.3
200	102.6	243	125.5	286	148.8	329	172.5	372	196.8
201	103.1	244	126.0	287	149.5	330	173.1	373	197.4
202	103.7	245	126.6	288	149.4	331	173.7	374	198.0
203	104.2	246	127.1	289	150.9	332	174.2	375	198.6
204	104.7	247	127.6	290	151.0	333	174.8	376	199.1
205	105.3	248	128.1	291	151.6	334	175.3	377	199.7
206	105.8	249	128.7	292	152.1	335	175.9	378	200.3
207	106.3	250	129.2	293	152.7	336	176.5	379	200.8
208	106.8	251	129.7	294	153.2	337	177.0	380	201.4
209	107.4	252	130.3	295	153.8	338	177.6	381	202.0
210	107.9	253	130.8	296	154.3	339	178.1	382	202.5
211	108.4	254	131.4	297	154.9	340	178.7	383	203.1
212	109.0	255	131.9	298	155.4	341	179.3	384	203.7
213	109.5	256	132.4	299	156.0	342	179.8	385	204.3
214	110.0	257	133.0	300	156.5	343	180.4	386	204.8
215	110.6	258	133.5	301	157.1	344	180.9	387	205.4
216	111.1	259	134.1	302	157.6	345	181.5	388	206.0
217	111.6	260	134.6	303	158.2	346	182.1	389	206.5
218	112.1	261	135.1	304	158.7	347	182.6	390	207.1
219	112.7	262	135.7	305	159.3	348	182.3	391	207.7
220	113.2	263	136.2	306	159.8	349	183.7	392	208.3
221	113.7	264	136.8	307	160.4	350	184.3	393	208.8
222	114.3	265	137.3	308	160.9	351	184.9	394	209.4
223	114.8	266	137.8	309	161.5	352	185.4	395	210.0
224	115.3	267	138.4	310	162.0	353	186.0	396	210.6
225	115.9	268	138.9	311	162.6	354	186.6	397	211.2
226	116.4	269	139.5	312	163.1	355	187.2	398	211.7
227	116.9	270	140.0	313	163.7	356	187.7	399	212.3
228	117.4	271	140.6	314	164.2	357	188.3	400	212.9
229	118.0	272	141.1	315	164.8	358	188.9	401	213.5
230	118.5	273	141.7	316	165.3	359	189.4	402	214.1
231	119.0	274	142.2	317	165.9	360	190.0	403	214.6
232	119.6	275	142.8	318	166.4	361	190.6	404	215.2

Milli-grams of cop-per	Milli-grams of dex-trose	Milli-grams of cop-per	Milli-grams of dex-trose	Milli-grams of cop-per	Milli-grams of dex-trose	Milli-grams of cop-per	Milli-grams of dex-trose	Milli-grams of cop-per	Milli-grams of dex-trose
405	215.8	417	222.8	429	229.8	441	236.9	453	244.0
406	216.4	418	223.3	430	230.4	442	237.5	454	244.6
407	217.0	419	223.9	431	231.0	443	238.1	455	245.2
408	217.5	420	224.5	432	231.6	444	238.7	456	245.7
409	218.1	421	225.1	433	232.2	445	239.3	457	246.3
410	218.7	422	225.7	434	232.8	446	239.8	458	246.9
411	219.3	423	226.3	435	233.4	447	240.4	459	247.5
412	219.9	424	226.9	436	233.9	448	241.0	460	248.1
413	220.4	425	227.5	437	234.5	449	241.6	461	248.7
414	221.0	426	228.0	438	235.1	450	242.2	462	249.3
415	221.6	427	228.6	439	235.7	451	242.8	463	249.9
416	222.2	428	229.2	440	236.3	452	244.4		

180. Preparation of Levulose.—It is not often that levulose, unmixed with other reducing sugars, is brought to the attention of the analyst. It probably does not exist in the unmixed state in many agricultural products. The easiest method of preparing it is by the hydrolysis of inulin. A nearly pure levulose has also lately been placed on the market under the name of 'diabetin'. It is prepared from invert sugar.

Inulin is prepared from dahlia bulbs by boiling the pulp with water and a trace of calcium carbonate. The extract is concentrated to a sirup and subjected to a freezing temperature to promote the crystallization of the inulin. The separated product is subjected to the above operations several times until it is pure and colorless. It is then washed with alcohol and ether and is reduced to a fine powder. Before the repeated treatment with water it is advisable to clarify the solution with lead subacetate. The lead is afterwards removed by hydrogen sulfid and the resultant acetic acid neutralized with calcium carbonate.

By the action of hot dilute acids inulin is rapidly converted into levulose.

Levulose may also be prepared from invert sugar, but in this case it is difficult to free it from traces of dextrose. The most successful method consists in forming a lime compound with the

invert sugar and separating the lime levulosate and dextrosate by their difference in solubility. The levulose salt is much less soluble than the corresponding compound of dextrose. In the manufacture of levulose from beet molasses, the latter is dissolved in six times its weight of water and inverted with a quantity of hydrochloric acid, proportioned to the quantity of ash present in the sample. After inversion the mixture is cooled to zero and the levulose precipitated by adding fine-ground lime. The dextrose and coloring matters in these conditions are not thrown down. The precipitated lime levulosate is separated by filtration and washed with ice-cold water. The lime salt is afterwards beaten to a cream with water and decomposed by carbon dioxide. The levulose, after filtration, is concentrated to the crystallizing point.⁸⁸

181. Estimation of Levulose.—Levulose, when free of any admixture with other reducing sugars, may be determined by the copper method with the use of the subjoined table, prepared by Lehmann.⁸⁹ The copper solution is the same as that used for invert sugar, *viz.*, 69.278 grams of pure copper sulfate in one liter. The alkali solution is prepared by dissolving 346 grams of Rochelle salt and 250 grams of sodium hydroxid in water and completing the volume to one liter.

Manipulation.—Twenty-five cubic centimeters of each solution are mixed with 50 of water and boiled. To the boiling mixture 25 cubic centimeters of the levulose solution are added, which must not contain more than one per cent. of the sugar. The boiling is then continued for 15 minutes, and the cuprous oxide collected, washed and reduced to the metallic state in the usual way. The quantity of levulose is then determined by inspection from the table given below. Other methods of determining levulose in mixtures will be given further on.

⁸⁸ Chemiker-Zeitung, 1893, 17: 548.

⁸⁹ Wein, Tabellen zur quantitativen Bestimmung der Zuckerarten 1888: 35.

TABLE FOR THE ESTIMATION OF LEVULOSE.

Milli-grams copper	Milli-grams levulose	Milli-grams copper	Milli-grams levulose	Milli-grams copper	Milli-grams levulose	Milli-grams copper	Milli-grams levulose
20	7.15	62	31.66	104	56.85	146	82.81
21	7.78	63	32.25	105	57.46	147	83.43
22	8.41	64	32.84	106	58.07	148	84.06
23	9.04	65	33.43	107	58.68	149	84.68
24	9.67	66	34.02	108	59.30	150	85.31
25	10.30	67	34.62	109	59.91	151	85.93
26	10.81	68	35.21	110	60.52	152	86.55
27	11.33	69	35.81	111	61.13	153	87.16
28	11.84	70	36.40	112	61.74	154	87.88
29	12.36	71	37.00	113	62.36	155	88.40
30	12.87	72	37.59	114	62.97	156	89.05
31	13.46	73	38.19	115	63.58	157	89.69
32	14.05	74	38.78	116	64.21	158	90.34
33	14.64	75	39.38	117	64.84	159	90.98
34	15.23	76	39.98	118	65.46	160	91.63
35	15.82	77	40.58	119	66.07	161	92.26
36	16.40	78	41.17	120	66.72	162	92.90
37	16.99	79	41.77	121	67.32	163	93.53
38	17.57	80	42.37	122	67.92	164	94.17
39	18.16	81	42.97	123	68.53	165	94.80
40	18.74	82	43.57	124	69.13	166	95.44
41	19.32	83	44.16	125	69.73	167	96.08
42	19.91	84	44.76	126	70.35	168	96.77
43	20.49	85	45.30	127	70.96	169	97.33
44	21.08	86	45.96	128	71.58	170	97.99
45	21.66	87	46.57	129	72.19	171	98.63
46	22.25	88	47.17	130	72.81	172	99.27
47	22.83	89	47.78	131	73.43	173	99.90
48	23.42	90	48.38	132	74.05	174	100.54
49	24.00	91	48.98	133	74.67	175	101.18
50	24.59	92	49.58	134	75.29	176	101.82
51	25.18	93	50.18	135	75.91	177	102.46
52	25.76	94	50.78	136	76.53	178	103.11
53	26.35	95	51.38	137	77.15	179	103.75
54	26.93	96	51.98	138	77.77	180	104.39
55	27.52	97	52.58	139	78.39	181	105.04
56	28.11	98	53.19	140	79.01	182	105.68
57	28.70	99	53.79	141	79.64	183	106.33
58	29.30	100	54.39	142	80.28	184	106.97
59	29.89	101	55.00	143	80.91	185	107.62
60	30.48	102	55.62	144	81.55	186	108.27
61	31.07	103	56.23	145	82.18	187	108.92

TABLE FOR THE ESTIMATION OF LEVULOSE.—(Continued.)

Milli-grams copper	Milli-grams levulose	Milli-grams copper	Milli-grams levulose	Milli-grams copper	Milli-grams levulose	Milli-grams copper	Milli-grams levulose
188	109.56	232	138.57	276	168.68	320	199.97
189	110.21	233	139.25	277	169.37	321	200.71
190	110.86	234	139.18	278	170.06	322	201.44
191	111.50	235	140.59	279	170.75	323	202.18
192	112.14	236	141.27	280	171.44	324	202.91
193	112.78	237	141.94	281	172.14	325	203.65
194	113.42	238	142.62	282	172.85	326	204.39
195	114.06	239	143.29	283	173.55	327	205.13
196	114.72	240	143.97	284	174.26	328	205.88
197	115.38	241	144.65	285	174.96	329	206.62
198	116.04	242	145.32	286	175.67	330	207.36
199	116.70	243	146.00	287	176.39	331	208.10
200	117.36	244	146.67	288	177.10	332	208.83
201	118.02	245	147.35	289	177.82	333	209.57
202	118.68	246	148.03	290	178.53	334	210.30
203	119.33	247	148.71	291	179.24	335	211.04
204	119.99	248	149.40	292	179.95	336	211.78
205	120.65	249	150.08	293	180.65	337	212.52
206	121.30	250	150.76	294	181.33	338	213.25
207	121.96	251	151.44	295	182.07	339	213.99
208	122.61	252	152.12	296	182.78	340	214.73
209	123.27	253	152.81	297	183.49	341	215.48
210	123.92	254	153.49	298	184.21	342	216.23
211	124.58	255	154.17	299	184.92	343	216.97
212	125.24	256	154.91	300	185.63	344	217.72
213	125.90	257	155.65	301	186.35	345	218.47
214	126.56	258	156.40	302	187.06	346	218.21
215	127.22	259	157.14	303	187.78	347	219.07
216	127.85	260	157.88	304	188.49	348	220.71
217	128.48	261	158.49	305	189.21	349	221.46
218	129.10	262	159.09	306	189.93	350	222.21
219	129.73	263	159.70	307	190.65	351	222.96
220	130.36	264	160.30	308	191.37	352	223.72
221	131.07	265	160.91	309	192.09	353	224.47
222	131.77	266	161.63	310	192.81	354	225.23
223	132.48	267	162.35	311	193.53	355	225.98
224	133.18	268	163.07	312	194.25	356	226.74
225	133.89	269	163.79	313	194.97	357	227.49
226	134.56	270	164.51	314	195.69	358	228.25
227	135.23	271	165.21	315	196.41	359	229.00
228	135.89	272	165.90	316	197.12	360	229.76
229	136.89	273	166.60	317	197.83	361	230.52
230	137.23	274	167.29	318	198.55	362	231.28
231	137.90	275	167.99	319	199.26	363	232.05

182. Meissl's Table for Invert Sugar.—Invert sugar is usually the product of the hydrolysis of sucrose. The following table is to be used when the hydrolysis is complete, *i. e.*, when no sucrose

TABLE FOR INVERT SUGAR BY MEISSL AND WIEN.*

Milli-grams of cop-per	Milli-grams of invert sugar	Milli-grams of cop-per	Milli-grams of invert sugar	Milli-grams of cop-per	Milli-grams of invert sugar	Milli-grams of cop-per	Milli-grams of invert sugar
90	46.9	133	69.7	176	93.0	219	117.0
91	47.4	134	70.3	177	93.5	220	117.5
92	47.9	135	70.8	178	94.1	221	118.1
93	48.4	136	71.3	179	94.6	222	118.7
94	48.9	137	71.9	180	95.2	223	119.2
95	49.5	138	72.4	181	95.7	224	119.8
96	50.0	139	72.9	182	96.2	225	120.4
97	50.5	140	73.5	183	96.8	226	120.9
98	51.1	141	74.0	184	97.3	227	121.5
99	51.6	142	74.5	185	97.8	228	122.1
100	52.1	143	75.1	186	98.4	229	122.6
101	52.7	144	75.6	187	99.0	230	123.2
102	53.2	145	76.1	188	99.5	231	123.8
103	53.7	145	76.7	189	100.1	232	124.3
104	54.3	147	77.2	190	100.6	233	124.9
105	54.8	148	77.8	191	101.2	234	125.5
106	55.3	149	78.3	192	101.7	235	126.0
107	55.9	150	78.9	193	102.3	236	126.6
108	56.4	151	79.4	194	102.9	237	127.2
109	56.9	152	80.0	195	103.4	238	127.8
110	57.5	153	80.5	196	104.0	239	128.3
111	58.0	154	81.0	197	104.6	240	128.9
112	58.5	155	81.6	198	105.1	241	129.5
113	59.1	156	82.1	199	105.7	242	130.0
114	59.6	157	82.7	200	106.3	243	130.6
115	60.1	158	83.2	201	106.8	244	131.2
116	60.7	159	83.8	202	107.4	245	131.8
117	61.2	160	84.3	203	107.9	246	132.3
118	61.7	161	84.8	204	108.5	247	132.9
119	62.3	162	85.4	205	109.1	248	133.5
120	62.8	163	85.9	206	109.6	249	134.1
121	63.3	164	86.5	207	110.2	250	134.6
122	63.9	165	87.0	208	110.8	251	135.2
123	64.4	166	87.6	209	111.3	252	135.8
124	64.9	167	88.1	210	111.9	253	136.3
125	65.5	168	88.6	211	112.5	254	136.9
126	66.0	169	89.2	212	113.0	255	137.5
127	66.5	170	89.7	213	113.6	256	138.1
128	67.1	171	90.3	214	114.2	257	138.6
129	67.6	172	90.8	215	114.7	258	139.2
130	68.1	173	91.4	216	115.3	259	139.8
131	68.7	174	91.9	217	115.8	260	140.4
132	69.2	175	92.4	218	116.4	261	140.9

* Wein, Tabellen zur quantitativen Bestimmung der Zuckerarten, 1888 :

TABLE FOR INVERT SUGAR BY MEISSL AND WIEN.—(Continued.)

Milli-grams of cop- per	Milli-grams of invert sugar	Milli-grams of cop- per	Milli-grams of invert sugar	Milli-grams of cop- per	Milli-grams of invert sugar	Milli-grams of cop- per	Milli-grams of invert sugar
262	141.5	305	166.8	348	192.6	391	219.3
263	142.1	306	167.3	349	193.2	392	219.9
264	142.7	307	167.9	350	193.8	393	220.5
265	143.2	308	168.5	351	194.4	394	221.2
266	143.8	309	169.1	352	195.0	395	221.8
267	144.4	310	169.7	353	195.6	396	222.4
268	144.9	311	170.3	354	196.2	397	223.1
269	145.5	312	170.9	355	196.8	398	223.7
270	146.1	313	171.5	356	197.4	399	224.3
271	146.7	314	172.1	357	198.0	400	224.9
272	147.2	315	172.7	358	198.6	401	225.7
273	147.8	316	173.3	359	199.2	402	226.4
274	148.4	317	173.9	360	199.8	403	227.1
275	149.0	318	174.5	361	200.4	404	227.8
276	149.5	319	175.1	362	201.1	405	228.6
277	150.1	320	175.6	363	201.7	406	229.3
278	150.7	321	176.2	364	202.3	407	230.0
279	151.3	322	176.8	365	203.0	408	230.7
280	151.9	323	177.4	366	203.6	409	231.4
281	152.5	324	178.0	367	204.2	410	232.1
282	153.1	325	178.6	368	204.8	411	232.8
283	153.7	326	179.2	369	205.5	412	233.5
284	154.3	327	179.8	370	206.1	413	234.3
285	154.9	328	180.4	371	206.7	414	235.0
286	155.5	329	181.0	372	207.3	415	235.7
287	156.1	330	181.6	373	208.0	416	236.4
288	156.7	331	182.2	374	208.6	417	237.1
289	157.2	332	182.8	375	209.2	418	237.8
290	157.8	333	183.5	376	209.9	419	238.5
291	158.4	334	184.1	377	210.5	420	239.2
292	159.0	335	184.7	378	211.1	421	239.9
293	159.6	336	185.4	379	211.7	422	240.6
294	160.2	337	186.0	380	212.4	423	241.3
295	160.8	338	186.6	381	213.0	424	242.0
296	161.4	339	187.2	382	213.6	425	242.7
297	162.0	340	187.8	383	214.3	426	243.4
298	162.6	341	188.4	384	214.9	427	244.1
299	163.2	342	189.0	385	215.5	428	244.9
300	163.8	343	189.6	386	216.1	429	245.6
301	164.4	344	190.2	387	216.8	430	246.3
302	165.0	345	190.8	388	217.4		
303	165.6	346	191.4	389	218.0		
304	166.2	347	192.0	390	218.7		

is left in the solution. The solution of copper sulfate and of the alkaline tartrate are made up as follows: 34.64 grams of copper sulfate in half a liter, and 173 grams of Rochelle salt and 51.6 grams sodium hydroxid in the same volume. The quantity of sugar solution used must not contain more than 245 nor less than 90 milligrams of invert sugar.

In the determination 25 cubic centimeters of the copper solution and an equal volume of the alkaline tartrate are mixed and boiled, the proper amount of sugar solution added to secure a quantity of copper within the limits named, the volume completed to 100 cubic centimeters with boiling water, and the mixture kept in lively ebullition for two minutes. An equal volume of recently boiled cold water is added and the cuprous oxid at once separated by filtration on asbestos under pressure, and washed free of alkali with boiling water. The metallic copper is secured by one of the methods already described.

183. Meissl and Hiller's Method for More Than one Per cent. of Invert Sugar.⁹⁰—Prepare a solution of the material to be examined in such a manner that it contains 20 grams of the mixed sugars in 100 cubic centimeters, after clarification and the removal of the excess of lead. Prepare a series of solutions in large test tubes by adding one, two, three, four, five, etc., cubic centimeters of this solution to each tube successively. Add five cubic centimeters of the mixed copper reagent after Soxhlet formula to each, heat to boiling, boil two minutes and filter. Note the volume of sugar solution which gives the filtrate lightest in tint, but still distinctly blue. Place 20 times this volume of the sugar solution in a 100 cubic centimeter flask, dilute to the mark, and mix well. Use 50 cubic centimeters of the solution for the determination, which is conducted as already described under Herzfeld's method until the weight of copper is obtained. For the calculation of the results use the following formulas and table of factors of Meissl and Hiller.⁹¹

⁹⁰ This is used in cases of over 1% of invert sugar in samples of commercial sugars.

⁹¹ *Zeitschrift des Vereins für die Rübenzucker-Industrie*, 1889, **39**, *Neuer folge*, **26**: 735.

Let Cu = the weight of the copper obtained;

P = the polarization of the sample;

W = the weight of the sample in the 50 cubic centimeters of the solution used for determination;

F = the factor obtained from the table for conversion of copper to invert sugar;

$\frac{\text{Cu}}{2}$ = approximate absolute weight of invert sugar = Z;

$Z \times \frac{100}{W}$ = approximate per cent. of invert sugar = y;

$\frac{100 P}{P + y}$ = R, relative number for sucrose;

100 - R = I, relative number for invert sugar;

$\frac{\text{Cu} F}{W}$ = per cent. of invert sugar.

Z indicates the vertical column, see table p. 220; and the ratio of R to I, the horizontal column of the table which are to be used for the purpose of finding the factor (F) for calculating copper to invert sugar.

Example:—The polarization of a sugar is 86.4, and 3.256 grams of it (W) are equivalent to 0.290 gram of copper. Then:

$$\frac{\text{Cu}}{2} = \frac{0.290}{2} = 0.145 = Z.$$

$$Z \times \frac{100}{W} = 0.145 \times \frac{100}{3.256} = 4.45 = y.$$

$$\frac{100 P}{P + y} = \frac{86.40}{86.4 + 4.45} = 95.1 = R.$$

$$100 - R = 100 - 95.1 = 4.9 = I.$$

$$R : I = 95.1 : 4.9.$$

By consulting the table it will be seen that the vertical column headed 150 is nearest to Z, 145, the horizontal column headed

95 : 5 is nearest to the ratio of R to I, 95.1 : 4.9. Where these columns meet we find the factor 51.2, which enters into the final calculation :

$$\frac{\text{Cu F}}{\text{W}} = \frac{.290 \times 51.2}{3.256} = 4.56 \text{ the true per cent. of invert sugar.}$$

MEISSL AND HILLER'S FACTORS FOR DETERMINATIONS IN MATERIALS IN WHICH, OF THE TOTAL SUGARS PRESENT, 1 PER CENT. OR MORE IS INVERT SUGAR, AND 99 PER CENT. OR LESS IS SUCROSE.

Ratio of sucrose to invert sugar - R:I	Approximate absolute weight of invert sugar - Z						
	200 milli- grams Percent.	175 milli- grams Percent.	150 milli- grams Percent.	125 milli- grams Percent.	100 milli- grams Percent.	75 milli- grams Percent.	50 milli- grams Percent.
0:100	56.4	55.4	54.5	53.8	53.2	53.0	53.0
10:90	56.3	55.3	54.4	53.8	53.2	52.9	52.9
20:80	56.2	55.2	54.3	53.7	53.2	52.7	52.7
30:70	56.1	55.1	54.2	53.7	53.2	52.6	52.6
40:60	55.9	55.0	54.1	53.6	53.1	52.5	52.4
50:50	55.7	54.9	54.0	53.5	53.1	52.3	52.2
60:40	55.6	54.7	53.8	53.2	52.8	52.1	51.9
70:30	55.5	54.5	53.5	52.9	52.5	51.9	51.6
80:20	55.4	54.3	53.3	52.7	52.2	51.7	51.3
90:10	54.6	53.6	53.1	52.6	52.1	51.6	51.2
91:9	54.1	53.6	52.6	52.1	51.6	51.2	50.7
92:8	53.6	53.1	52.1	51.6	51.2	50.7	50.3
93:7	53.6	53.1	52.1	51.2	50.7	50.3	49.8
94:6	53.1	52.6	51.6	50.7	50.3	49.8	48.9
95:5	52.6	52.1	51.2	50.3	49.4	48.9	48.5
96:4	52.1	51.2	50.7	49.8	48.9	47.7	46.9
97:3	50.7	50.3	49.8	48.9	47.7	46.2	45.1
98:2	49.9	48.9	48.5	47.3	45.8	43.3	40.0
99:1	47.7	47.3	46.5	45.1	43.3	41.2	38.1

184. Soxhlet Method for Lactose.—This method is used to some extent by chemists in the determination of lactose in milk. However, its use is being supplanted by that of Munson and Walker given below, so very little space will be given to it.

In the conduct of the work 25 cubic centimeters of the copper solution are mixed with an equal quantity of the alkaline tartrate mixture after Soxhlet's formula, and from 20 to 100 cubic centimeters of the sugar solution added, according to its con-

centration. This solution should not contain less than seventy nor more than 306 milligrams of lactose, but preferably nearer the higher limit. The volume is completed to 150 cubic centimeters with boiling water and kept in boiling for six minutes. The amount of copper may be determined by the reduction method given above (p. 203) or by any of the methods given later (p. 225). From the weight of copper obtained the quantity of milk sugar is determined by inspecting the table. It is recommended to use such a weight of milk sugar as will give about 200 milligrams of copper.

TABLE FOR THE DETERMINATION OF LACTOSE (SOXHLET-WEIN).

Milli-grams of copper	Milli-grams of lactose	Milli-grams of copper	Milli-grams of lactose	Milli-grams of copper	Milli-grams of lactose	Milli-grams of copper	Milli-grams of lactose	Milli-grams of copper	Milli-grams of lactose
100	71.6	125	90.1	150	108.8	175	127.8	200	146.9
101	72.4	126	90.9	151	109.6	176	128.5	201	147.7
102	73.1	127	91.6	152	110.3	177	129.3	202	148.5
103	73.8	128	92.4	153	111.1	178	130.1	203	149.2
104	74.6	129	93.1	154	111.9	179	130.8	204	150.0
105	75.3	130	93.8	155	112.6	180	131.6	205	150.7
106	76.1	131	94.6	156	113.4	181	132.4	206	151.5
107	76.8	132	95.3	157	114.1	182	133.1	207	152.2
108	77.6	133	96.1	158	114.9	183	133.9	208	153.0
109	78.3	134	96.9	159	115.6	184	134.7	209	153.7
110	79.0	135	97.6	160	116.4	185	135.4	210	154.5
111	79.8	136	98.3	161	117.1	186	136.2	211	155.2
112	80.5	137	99.1	162	117.9	187	137.0	212	156.0
113	81.3	138	99.8	163	118.6	188	137.7	213	156.7
114	82.0	139	100.5	164	119.4	189	138.5	214	157.5
115	82.7	140	101.3	165	120.2	190	139.3	215	158.2
116	83.5	141	102.0	166	120.9	191	140.0	216	159.0
117	84.2	142	102.8	167	121.7	192	140.8	217	159.7
118	85.0	143	103.5	168	122.4	193	141.6	218	160.4
119	85.7	144	104.3	169	123.2	194	142.3	219	161.2
120	86.4	145	105.1	170	123.9	195	143.1	220	161.9
121	87.2	146	105.8	171	124.7	196	143.9	221	162.7
122	87.9	147	106.6	172	125.5	197	144.6	222	163.4
123	88.7	148	107.3	173	126.2	198	145.4	223	164.2
124	89.4	149	108.1	174	127.0	199	146.2	224	164.9

TABLE FOR THE DETERMINATION OF LACTOSE (SOXHLET-WEIN)—Cont'd.

Milli-grams of cop-per	Milli-grams of lac-tose	Milli-grams of cop-per	Milli-grams of lac-tose	Milli-grams of cop-per	Milli-grams of lac-tose	Milli-grams of cop-per	Milli-grams of lac-tose	Milli-grams of cop-per	Milli-grams of lac-tose
225	165.7	261	193.3	297	221.9	333	250.0	369	279.6
226	166.4	262	194.1	298	222.7	334	250.8	370	280.5
227	167.2	263	194.9	299	223.5	335	251.6	371	281.4
228	167.9	264	195.7	300	224.4	336	252.5	372	282.2
229	168.6	265	196.4	301	225.2	337	253.3	373	283.1
230	169.4	266	197.2	302	225.9	338	254.1	374	283.9
231	170.1	267	198.0	303	226.7	339	254.9	375	284.8
232	170.9	268	198.8	304	227.5	340	255.7	376	285.7
233	171.6	269	199.5	305	228.3	341	256.5	377	286.5
234	172.4	270	200.3	306	229.1	342	257.4	378	287.4
235	173.1	271	201.1	307	229.8	343	258.2	379	288.2
236	173.9	272	201.9	308	230.6	344	259.0	380	289.1
237	174.6	273	202.7	309	231.4	345	259.8	381	289.9
238	175.4	274	203.5	310	232.2	346	260.6	382	290.8
239	176.2	275	204.3	311	231.9	347	261.4	383	291.7
240	176.9	276	205.1	312	233.7	348	262.3	384	292.5
241	177.7	277	205.9	313	234.5	349	263.1	385	293.4
242	178.5	278	206.7	314	235.3	350	263.9	386	294.2
243	179.3	279	207.5	315	236.1	351	264.7	387	295.1
244	180.1	280	208.3	316	236.8	352	265.5	388	296.0
245	180.8	281	209.1	317	237.6	353	266.3	389	296.8
246	181.6	282	209.9	318	238.4	354	267.2	390	297.7
247	182.4	283	210.7	319	239.2	355	268.0	391	298.5
248	183.2	284	211.5	320	240.0	356	268.8	392	299.4
249	184.0	285	212.3	321	240.7	357	269.6	393	300.3
250	184.8	286	213.1	322	241.5	358	270.4	394	301.1
251	185.5	287	213.9	323	242.3	359	271.2	395	302.0
252	186.3	288	214.7	324	243.1	360	272.1	396	302.8
253	187.1	289	215.5	325	243.9	361	272.9	397	303.7
254	187.9	290	216.3	326	244.6	362	273.7	398	304.6
255	188.7	291	217.1	327	245.4	363	274.5	399	305.4
256	189.4	292	217.9	328	246.2	364	275.3	400	306.4
257	190.2	293	218.7	329	247.0	365	276.2		
258	191.0	294	219.5	330	247.7	366	277.1		
259	191.8	295	220.3	331	248.5	367	277.9		
260	192.5	296	221.1	332	249.2	368	278.8		

185. Wein's Method for Maltose.—Like the former method, this still finds many users. The procedure is as follows, Soxhlet's solution is used in the determination.

Place 50 cubic centimeters of the mixed copper reagent in a beaker and heat to the boiling point. While boiling briskly add 25 cubic centimeters of the maltose solution containing not more than 0.250 gram of maltose and boil for four minutes. Filter immediately through asbestos and determine the amount of copper reduced by one of the methods given later. Obtain the weight of maltose equivalent to the weight of copper found from the following table:

TABLE FOR THE DETERMINATION OF MALTOSE.

[According to Wein.]

	Milligrams of copper	Milligrams of cuprous oxid	Milligrams of maltose	Milligrams of copper	Milligrams of cuprous oxid	Milligrams of maltose	Milligrams of copper	Milligrams of cuprous oxid	Milligrams of maltose	Milligrams of copper	Milligrams of cuprous oxid	Milligrams of maltose
31	34.9	26.1	56	63.0	47.8	81	91.2	69.7	106	119.3	91.9	
32	36.0	27.0	57	64.2	48.7	82	92.3	70.6	107	120.5	92.8	
33	37.2	27.9	58	65.3	49.6	83	93.4	71.5	108	121.6	93.7	
34	38.3	28.7	59	66.4	50.4	84	94.6	72.4	109	122.7	94.6	
35	39.4	29.6	60	67.6	51.3	85	95.7	73.2	110	123.8	95.5	
36	40.5	30.5	61	68.7	52.2	86	96.8	74.1	111	125.0	96.4	
37	41.7	31.3	62	69.8	53.1	87	97.9	75.0	112	126.1	97.3	
38	42.8	32.2	63	70.9	53.9	88	99.1	75.9	113	127.2	98.1	
39	43.9	33.1	64	72.1	54.8	89	100.2	76.8	114	128.3	99.0	
40	45.0	33.9	65	73.2	55.7	90	101.3	77.7	115	129.6	99.9	
41	46.2	34.8	66	74.3	56.6	91	102.4	78.6	116	130.6	100.8	
42	47.3	35.7	67	75.4	57.4	92	103.6	79.5	117	131.7	101.7	
43	48.4	36.5	68	76.6	58.3	93	104.7	80.3	118	132.8	102.6	
44	49.5	37.4	69	77.7	59.2	94	105.8	81.2	119	134.0	103.5	
45	50.7	38.3	70	78.8	60.1	95	107.0	82.1	120	135.1	104.4	
46	51.8	39.1	71	79.9	61.0	96	108.1	83.0	121	136.2	105.3	
47	52.9	40.0	72	81.1	61.8	97	109.2	83.9	122	137.4	106.2	
48	54.0	40.9	73	82.2	62.7	98	110.3	84.8	123	138.5	107.1	
49	55.2	41.8	74	83.3	63.6	99	111.5	85.7	124	139.6	108.0	
50	56.3	42.6	75	84.4	64.5	100	112.6	86.6	125	140.7	108.9	
51	57.4	43.5	76	85.6	65.4	101	113.7	87.5	126	141.9	109.8	
52	58.5	44.4	77	86.7	66.2	102	114.8	88.4	127	143.0	110.7	
53	59.7	45.2	78	87.8	67.1	103	116.0	89.2	128	144.1	111.6	
54	60.8	46.1	79	88.9	68.0	104	117.1	90.1	129	145.2	112.5	
55	61.9	47.0	80	90.1	68.9	105	118.2	91.0	130	146.4	113.4	

TABLE FOR THE DETERMINATION OF MALTOSE—(Continued).

Milligrams of copper	Milligrams of cuprous oxid	Milligrams of maltose	Milligrams of copper	Milligrams of cuprous oxid	Milligrams of maltose	Milligrams of copper	Milligrams of cuprous oxid	Milligrams of maltose	Milligrams of copper	Milligrams of cuprous oxid	Milligrams of maltose
131	147.5	114.3	176	198.1	154.7	221	248.7	194.8	266	299.5	235.2
132	148.6	115.2	177	199.3	155.6	222	249.9	195.7	267	300.6	236.1
133	149.7	116.1	178	200.4	156.5	223	251.0	196.6	268	301.7	237.0
134	150.9	117.0	179	201.5	157.4	224	252.4	197.5	269	302.8	237.9
135	152.0	117.9	180	202.6	158.3	225	253.3	198.4	270	304.0	238.8
136	153.1	118.8	181	203.8	159.2	226	254.4	199.3	271	305.1	239.7
137	154.2	119.7	182	204.9	160.1	227	255.6	200.2	272	306.2	240.6
138	155.4	120.6	183	206.0	160.9	228	256.7	201.1	273	307.3	241.5
139	156.5	121.5	184	207.1	161.8	229	257.8	202.0	274	308.5	242.4
140	157.6	122.4	185	208.3	162.7	230	258.9	202.9	275	309.6	243.3
141	158.7	123.3	186	209.4	163.6	231	260.1	203.8	276	310.7	244.2
142	159.9	124.2	187	210.5	164.5	232	261.2	204.7	277	311.9	245.1
143	161.0	125.1	188	211.7	165.4	233	262.3	205.6	278	313.0	246.0
144	162.1	126.0	189	212.8	166.3	234	263.4	206.5	279	314.1	246.9
145	163.2	126.9	190	213.9	167.2	235	264.6	207.4	280	315.2	247.8
146	164.4	127.8	191	215.0	168.1	236	265.7	208.3	281	316.4	248.7
147	165.5	128.7	192	216.2	169.0	237	266.8	209.1	282	317.5	249.6
148	166.6	129.6	193	217.3	169.8	238	268.0	210.0	283	318.6	250.4
149	167.7	130.5	194	218.4	170.7	239	269.1	210.9	284	319.7	251.3
150	168.9	131.4	195	219.5	171.6	240	270.2	211.8	285	320.9	252.2
151	170.0	132.3	196	220.7	172.5	241	271.3	212.7	286	322.0	253.1
152	171.1	133.2	197	221.8	173.4	242	272.5	213.6	287	323.1	254.0
153	172.1	134.1	198	222.9	174.3	243	273.6	214.5	288	324.2	254.9
154	173.4	135.0	199	224.1	175.2	244	274.7	215.4	289	325.4	255.8
155	174.5	135.9	200	225.2	176.1	245	275.8	216.3	290	326.5	256.6
156	175.6	136.8	201	226.3	177.0	246	277.0	217.2	291	327.4	257.5
157	176.8	137.7	202	227.4	177.9	247	278.1	218.1	292	328.7	258.4
158	177.9	138.6	203	228.5	178.7	248	279.2	219.0	293	329.9	259.3
159	179.0	139.5	204	229.7	179.6	249	280.3	219.9	294	331.0	260.2
160	180.1	140.4	205	230.8	180.5	250	281.5	220.8	295	332.1	261.1
161	181.3	141.3	206	231.9	181.4	251	282.6	221.7	296	333.2	262.0
162	182.4	142.2	207	233.0	182.3	252	283.7	222.6	297	334.4	262.8
163	183.5	143.1	208	234.2	183.2	253	284.8	223.5	298	335.5	263.7
164	184.6	144.0	209	235.3	184.1	254	286.0	224.4	299	336.6	264.6
165	185.8	144.9	210	236.4	185.0	255	287.1	225.3	300	337.8	265.5
166	186.9	145.8	211	237.6	185.9	256	288.2	226.2			
167	188.0	146.7	212	238.7	186.8	257	289.3	227.1			
168	189.1	147.6	213	23.8	187.7	258	290.5	228.0			
169	190.3	148.5	214	240.9	188.6	259	291.6	228.9			
170	191.4	149.4	215	242.1	189.5	260	292.7	229.8			
171	192.5	150.3	216	243.2	190.4	261	293.8	230.7			
172	193.6	151.2	217	244.3	191.2	262	295.0	231.6			
173	194.8	152.0	218	245.4	192.1	263	296.1	232.5			
174	195.9	152.9	219	246.6	193.0	264	297.2	233.4			
175	197.0	153.8	220	247.7	193.9	265	298.3	234.3			

186. Munson and Walker's Methods and Tables for the Determination of Dextrose, Invert Sugar Alone, Invert Sugar in the Presence of Sucrose, Lactose and Maltose.⁹²—A modification of the methods of determining reducing sugars has been devised by Munson and Walker.⁹³ This process embodies the best points of all the usual methods and with its attached tables is recommended for general use.

PREPARATION OF REAGENTS.

(I) *Copper Sulfate Solution.* Dissolve 34.639 grams of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in water and dilute to 500 cubic centimeters.

(II) *Alkaline Tartrate Solution.* Dissolve 173 grams of Rochelle salts plus 50 grams of sodium hydroxid in water and dilute to 500 cubic centimeters.

DETERMINATION.

Transfer 25 cubic centimeters each of the copper and alkaline tartrate solutions to a 400 cubic centimeter Jena or nonsoluble beaker and add 50 cubic centimeters of reducing sugar solution, or, if a smaller volume of sugar solution be used, add water to make the final volume 100 cubic centimeters. Heat the beaker upon an asbestos gauze over a bunsen, so regulate the flame that boiling begins in four minutes, and continue the boiling for exactly two minutes. Keep the beaker covered with a watch-glass throughout the entire time of heating. Without diluting, filter the cuprous oxid at once on an asbestos felt in a porcelain gooch crucible, using suction. Wash the cuprous oxid thoroughly with water at a temperature of about 60° , then with 10 cubic centimeters of alcohol and finally with 10 cubic centimeters of ether. Dry for thirty minutes in a water oven at 100° , cool in a desiccator and weigh as cuprous oxid.

The number of milligrams of copper reduced by a given amount of reducing sugar differs when sucrose is present and when it is absent. In the tables following the absence of sucrose is assumed except in the two columns under invert sugar, where

⁹² Bureau of Chemistry Bull. 107 Revised: 242-251.

⁹³ Journal American Chemical Society, 1906, 28: 663; 1907, 29: 541.

one for mixtures of invert sugar and sucrose (0.4 gram of total sugar in 50 cubic centimeters of solution) and one for invert sugar and sucrose when the 50 cubic centimeters of solution contains two grams of total sugar are given, in addition to the column for invert sugar alone.

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR IN THE PRESENCE OF SUCROSE (0.4 GRAM AND 2 GRAMS TOTAL SUGAR), LACTOSE,* AND MALTOSE.

[Expressed in Milligrams.]

Cuprous oxid (Cu_2O)	Copper (Cu)	Dextrose ($\mu\text{glucose}$)	Invert sugar	Invert sugar and sucrose		Lactose $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$	Maltose $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$	Cuprous oxid (Cu_2O)
				0.4 gram total sugar	2 grams total sugar			
10	8.9	4.0	4.5	1.6	6.3	6.2	10
11	9.8	4.5	5.0	2.1	6.9	7.0	11
12	10.7	4.9	5.4	2.5	7.5	7.9	12
13	11.5	5.3	5.8	3.0	8.2	8.7	13
14	12.4	5.7	6.3	3.4	8.8	9.5	14
15	13.3	6.2	6.7	3.9	9.4	10.4	15
16	14.2	6.6	7.2	4.3	10.0	11.2	16
17	15.1	7.0	7.6	4.8	10.7	12.0	17
18	16.0	7.5	8.1	5.2	11.3	12.9	18
19	16.9	7.9	8.5	5.7	11.9	13.7	19
20	17.8	8.3	8.9	6.1	12.5	14.6	20
21	18.7	8.7	9.4	6.6	13.2	15.4	21
22	19.5	9.2	9.8	7.0	13.8	16.2	22
23	20.4	9.6	10.3	7.5	14.4	17.1	23
24	21.3	10.0	10.7	7.9	15.0	17.9	24
25	22.2	10.5	11.2	8.4	15.7	18.7	25
26	23.1	10.9	11.6	8.8	16.3	19.6	26
27	24.0	11.3	12.0	9.3	16.9	20.4	27
28	24.9	11.8	12.5	9.7	17.6	21.2	28
29	25.8	12.2	12.9	10.2	18.2	22.1	29
30	26.6	12.6	13.4	10.7	4.3	18.8	22.9	30
31	27.5	13.1	13.8	11.1	4.7	19.4	23.7	31
32	28.4	13.5	14.3	11.6	5.2	20.1	24.6	32
33	29.3	13.9	14.7	12.0	5.6	20.7	25.4	33

* The Lactose figures are from Straughn and Given table.

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR
IN THE PRESENCE OF SUCROSE (0.4 GRAM AND 2 GRAMS TOTAL
SUGAR), LACTOSE, AND MALTOSE.—(Continued.)
[Expressed in Milligrams.]

	Cuprous oxid (Cu_2O)	Copper (Cu)	Dextrose (<i>d</i> -glucose)	Invert sugar	Invert sugar and sucrose		Lactose $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$	Maltose $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$	Cuprous oxid (Cu_2O)
					0.4 gram total sugar	2 grams total sugar			
34		30.2	14.3	15.2	12.5	6.1	21.4	26.2	34
35		31.1	14.8	15.6	12.9	6.5	22.1	27.1	35
36		32.0	15.2	16.1	13.4	7.0	22.8	27.9	36
37		32.9	15.6	16.5	13.8	7.4	23.5	28.7	37
38		33.8	16.1	16.9	14.3	7.9	24.2	29.6	38
39		34.6	16.5	17.4	14.7	8.4	24.8	30.4	39
40		35.5	16.9	17.8	15.2	8.8	25.5	31.3	40
41		36.4	17.4	18.3	15.6	9.3	26.2	32.1	41
42		37.3	17.8	18.7	16.1	9.7	26.9	32.9	42
43		38.2	18.2	19.2	16.6	10.2	27.6	33.8	43
44		39.1	18.7	19.6	17.0	10.7	28.3	34.6	44
45		40.0	19.1	20.1	17.5	11.1	28.9	35.4	45
46		40.9	19.6	20.5	17.9	11.6	29.6	36.3	46
47		41.7	20.0	21.0	18.4	12.0	30.3	37.1	47
48		42.6	20.4	21.4	18.8	12.5	31.5	37.9	48
49		43.5	20.9	21.9	19.3	12.9	31.7	38.8	49
50		44.4	21.3	22.3	19.7	13.4	32.3	39.6	50
51		45.3	21.7	22.8	20.2	13.9	33.0	40.4	51
52		46.2	22.2	23.2	20.7	14.3	33.7	41.3	52
53		47.1	22.6	23.7	21.1	14.8	34.4	42.1	53
54		48.0	23.0	24.1	21.6	15.2	35.1	42.9	54
55		48.9	23.5	24.6	22.0	15.7	35.8	43.8	55
56		49.7	23.9	25.0	22.5	16.2	36.4	44.6	56
57		50.6	24.3	25.5	22.9	16.6	37.1	45.4	57
58		51.5	24.8	25.9	23.4	17.1	37.8	46.3	58
59		52.4	25.2	26.4	23.9	17.5	38.5	47.1	59
60		53.3	25.6	26.8	24.3	18.0	39.2	48.0	60
61		54.2	26.1	27.3	24.8	18.5	39.9	48.8	61
62		55.1	26.5	27.7	25.2	18.9	40.5	49.6	62
63		56.0	27.0	28.2	25.7	19.4	41.2	50.5	63
64		56.8	27.4	28.6	26.2	19.8	41.9	51.3	64
65		57.7	27.8	29.1	26.6	20.3	42.6	52.1	65

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR
IN THE PRESENCE OF SUCROSE (0.4 GRAM AND 2 GRAMS TOTAL
SUGAR), LACTOSE, AND MALTOSE.—(Continued.)

[Expressed in Milligrams.]

Cuprous oxid (Cu ₂ O)	Copper (Cu)	Dextrose (d-glucose)	Invert sugar	Invert sugar and sucrose		Lactose C ₁₂ H ₂₂ O ₁₁ + H ₂ O	Maltose C ₁₂ H ₂₂ O ₁₁ + H ₂ O	Cuprous oxid (Cu ₂ O)
				0.4 gram total sugar	2 grams total sugar			
66	58.6	28.3	29.5	27.1	20.8	43.3	53.0	66
67	59.5	28.7	30.0	27.5	21.2	44.0	53.8	67
68	60.4	29.2	30.4	28.0	21.7	44.7	54.6	68
69	61.3	29.6	30.9	28.5	22.2	45.3	55.5	69
70	62.2	30.0	31.3	28.9	22.6	46.0	56.3	70
71	63.1	30.5	31.8	29.4	23.1	46.7	57.1	71
72	64.0	30.9	32.3	29.8	23.5	47.4	58.0	72
73	64.8	31.4	32.7	30.3	24.0	48.1	58.8	73
74	65.7	31.8	33.2	30.8	24.5	48.8	59.6	74
75	66.6	32.2	33.6	31.2	24.9	49.4	60.5	75
76	67.5	32.7	34.1	31.7	25.4	50.1	61.3	76
77	68.4	33.1	34.5	32.1	25.9	50.8	62.1	77
78	69.3	33.6	35.0	32.6	26.3	51.5	63.0	78
79	70.2	34.0	35.4	33.1	26.8	52.2	63.8	79
80	71.1	34.4	35.9	33.5	27.3	52.9	64.6	80
81	71.9	34.9	36.3	34.0	27.7	53.6	65.5	81
82	72.8	35.3	36.8	34.5	28.2	54.2	66.3	82
83	73.7	35.8	37.3	34.9	28.6	54.9	67.1	83
84	74.6	36.2	37.7	35.4	29.1	55.6	68.0	84
85	75.5	36.7	38.2	35.8	29.6	56.3	68.8	85
86	76.4	37.1	38.6	36.3	30.0	57.0	69.7	86
87	77.3	37.5	39.1	36.8	30.5	57.7	70.5	87
88	78.2	38.0	39.5	37.2	31.0	58.3	71.3	88
89	79.1	38.4	40.0	37.7	31.4	59.0	72.2	89
90	79.9	38.9	40.4	38.2	31.9	59.7	73.0	90
91	80.8	39.3	40.9	38.6	32.4	60.4	73.8	91
92	81.7	39.8	41.4	39.1	32.8	61.1	74.7	92
93	82.6	40.2	41.8	39.6	33.3	61.8	75.5	93
94	83.5	40.6	42.3	40.0	33.8	62.5	76.3	94
95	84.4	41.1	42.7	40.5	34.2	63.2	77.2	95
96	85.3	41.5	43.2	41.0	34.7	63.8	78.0	96
97	86.2	42.0	43.7	41.4	35.2	64.5	78.8	97

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR
IN THE PRESENCE OF SUCROSE (0.4 GRAM AND 2 GRAMS TOTAL
SUGAR), LACTOSE, AND MALTOSE.—(Continued.)

[Expressed in Milligrams.]

Cuprous oxid (Cu_2O)	Copper (Cu)	Dextrose (d -glucose)	Invert sugar	Invert sugar and sucrose		Lactose $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$	Maltose $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$	Cuprous oxid (Cu_2O)
				0.4 gram total sugar	2 grams total sugar			
98	87.1	42.4	44.1	41.9	35.6	65.2	79.7	98
99	87.9	42.9	44.6	42.4	36.1	65.9	80.5	99
100	88.8	43.3	45.0	42.8	36.6	66.6	81.3	100
101	89.7	43.8	45.5	43.3	37.0	67.3	82.2	101
102	90.6	44.2	46.0	43.8	37.5	68.0	83.0	102
103	91.5	44.7	46.4	44.2	38.0	68.7	83.8	103
104	92.4	45.1	46.9	44.7	38.5	69.3	84.7	104
105	93.3	45.5	47.3	45.2	38.9	70.0	85.5	105
106	94.2	46.0	47.8	45.6	39.4	70.7	86.3	106
107	95.0	46.4	48.3	46.1	39.9	71.4	87.2	107
108	95.9	46.9	48.7	46.6	40.3	72.1	88.0	108
109	96.8	47.3	49.2	47.0	40.8	72.8	88.8	109
110	97.7	47.8	49.6	47.5	41.3	73.5	89.7	110
111	98.6	48.2	50.1	48.0	41.7	74.2	90.5	111
112	99.5	48.7	50.6	48.4	42.2	74.8	91.3	112
113	100.4	49.1	51.0	48.9	42.7	75.5	92.2	113
114	101.3	49.6	51.5	49.4	43.2	76.2	93.0	114
115	102.2	50.0	51.9	49.8	43.6	76.9	93.9	115
116	103.0	50.5	52.4	50.3	44.1	77.6	94.7	116
117	103.9	50.9	52.9	50.8	44.6	78.3	95.5	117
118	104.8	51.4	53.3	51.2	45.0	79.0	96.4	118
119	105.7	51.8	53.8	51.7	45.5	79.6	97.2	119
120	106.6	52.3	54.3	52.2	46.0	80.3	98.0	120
121	107.5	52.7	54.7	52.7	46.5	81.0	98.9	121
122	108.4	53.2	55.2	53.1	46.9	81.7	99.7	122
123	109.3	53.6	55.7	53.6	47.4	82.4	100.5	123
124	110.1	54.1	56.1	54.1	47.9	83.1	101.4	124
125	111.0	54.5	56.6	54.5	48.3	83.8	102.2	125
126	111.9	55.0	57.0	55.0	48.8	84.5	103.0	126
127	112.8	55.4	57.5	55.5	49.3	85.1	103.9	127
128	113.7	55.9	58.0	55.9	49.8	85.8	104.7	128
129	114.6	56.3	58.4	56.4	50.2	86.5	105.5	129

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR
IN THE PRESENCE OF SUCROSE (0.4 GRAM AND 2 GRAMS TOTAL
SUGAR), LACTOSE, AND MALTOSE.—(Continued.)

[Expressed in Milligrams.]

Cuprous oxid (Cu_2O)	Copper (Cu)	Dextrose (α -glucose)	Invert sugar	Invert sugar and sucrose		Lactose $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$	Maltose $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$	Cuprous oxid (Cu_2O)
				0.4 gram total sugar	2 grams total sugar			
130	115.5	56.8	58.9	56.9	50.7	87.2	106.4	130
131	116.4	57.2	59.4	57.4	51.2	87.9	107.2	131
132	117.3	57.7	59.8	57.8	51.7	88.6	108.0	132
133	118.1	58.1	60.3	58.3	52.1	89.3	108.9	133
134	119.0	58.6	60.8	58.8	52.6	90.0	109.7	134
135	119.9	59.0	61.2	59.3	53.1	90.6	110.5	135
136	120.8	59.5	61.7	59.7	53.6	91.3	111.4	136
137	121.7	60.0	62.2	60.2	54.0	92.0	112.2	137
138	122.6	60.4	62.6	60.7	54.5	92.7	113.0	138
139	123.5	60.9	63.1	61.2	55.0	93.4	113.9	139
140	124.4	61.3	63.6	61.6	55.5	94.1	114.7	140
141	125.2	61.8	64.0	62.1	55.9	94.8	115.5	141
142	126.1	62.2	64.5	62.6	56.4	95.5	116.4	142
143	127.0	62.7	65.0	63.1	56.9	96.1	117.2	143
144	127.9	63.1	65.4	63.5	57.4	96.8	118.0	144
145	128.8	63.6	65.9	64.0	57.8	97.5	118.9	145
146	129.7	64.0	66.4	64.5	58.3	98.2	119.7	146
147	130.6	64.5	66.9	65.0	58.8	98.9	120.5	147
148	131.5	65.0	67.3	65.4	59.3	99.6	121.4	148
149	132.4	65.4	67.8	65.9	59.7	100.3	122.2	149
150	133.2	65.9	68.3	66.4	60.2	101.0	123.0	150
151	134.1	66.3	68.7	66.9	60.7	101.7	123.9	151
152	135.0	66.8	69.2	67.3	61.2	102.3	124.7	152
153	135.9	67.2	69.7	67.8	61.7	103.0	125.5	153
154	136.8	67.7	70.1	68.3	62.1	103.7	126.4	154
155	137.7	68.2	70.6	68.8	62.6	104.4	127.2	155
156	138.6	68.6	71.1	69.2	63.1	105.1	128.0	156
157	139.5	69.1	71.6	69.7	63.6	105.8	128.9	157
158	140.3	69.5	72.0	70.2	64.1	106.5	129.7	158
159	141.2	70.0	72.5	70.7	64.5	107.2	130.5	159
160	142.1	70.4	73.0	71.2	65.0	107.9	131.4	160
161	143.0	70.9	73.4	71.6	65.5	108.5	132.2	161

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR
IN THE PRESENCE OF SUCROSE (0.4 GRAM AND 2 GRAMS TOTAL
SUGAR), LACTOSE, AND MALTOSE.--(Continued.)
[Expressed in Milligrams.]

Cuprous oxid (Cu_2O)	Copper (Cu)	Dextrose (<i>d</i> -glucose)	Invert sugar	Invert sugar and sucrose		Lactose $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$	Maltose $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$	Cuprous oxid (Cu_2O)
				0.4 gram sugar total	2 grams sugar total			
162	143.9	71.4	73.9	72.1	66.0	109.2	133.0	162
163	144.8	71.8	74.4	72.6	66.5	109.9	133.9	163
164	145.7	72.3	74.9	73.1	66.9	110.6	134.7	164
165	146.6	72.8	75.3	73.6	67.4	111.3	135.5	165
166	147.5	73.2	75.8	74.0	67.9	112.0	136.4	166
167	148.3	73.7	76.3	74.5	68.4	112.7	137.2	167
168	149.2	74.1	76.8	75.0	68.9	113.4	138.0	168
169	150.1	74.6	77.2	75.5	69.3	114.1	138.9	169
170	151.0	75.1	77.7	76.0	69.8	114.8	139.7	170
171	151.9	75.5	78.2	76.4	70.3	115.4	140.5	171
172	152.8	76.0	78.7	76.9	70.8	116.1	141.4	172
173	153.7	76.4	79.1	77.4	71.3	116.8	142.2	173
174	154.6	76.9	79.6	77.9	71.7	117.5	143.0	174
175	155.5	77.4	80.1	78.4	72.2	118.2	143.9	175
176	156.3	77.8	80.6	78.8	72.7	118.9	144.7	176
177	157.2	78.3	81.0	79.3	73.2	119.6	145.5	177
178	158.1	78.8	81.5	79.8	73.7	120.3	146.4	178
179	159.0	79.2	82.0	80.3	74.2	121.0	147.2	179
180	159.9	79.7	82.5	80.8	74.6	121.7	148.0	180
181	160.8	80.1	82.9	81.3	75.1	122.3	148.9	181
182	161.7	80.6	83.4	81.7	75.6	123.0	149.7	182
183	162.6	81.1	83.9	82.2	76.1	123.7	150.5	183
184	163.4	81.5	84.4	82.7	76.6	124.4	151.4	184
185	164.3	82.0	84.9	83.2	77.1	125.1	152.2	185
186	165.2	82.5	85.3	83.7	77.6	125.8	153.0	186
187	166.1	82.9	85.8	84.2	78.0	126.5	153.9	187
188	167.0	83.4	86.3	84.6	78.5	127.2	154.7	188
189	167.9	83.9	86.8	85.1	79.0	127.9	155.5	189
190	168.8	84.3	87.2	85.6	79.5	128.5	156.4	190
191	169.7	84.8	87.7	86.1	80.0	129.2	157.2	191
192	170.5	85.3	88.2	86.6	80.5	129.9	158.0	192
193	171.4	85.7	88.7	87.1	81.0	130.6	158.9	193

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR
IN THE PRESENCE OF SUCROSE (0.4 GRAM AND 2 GRAMS TOTAL
SUGAR), LACTOSE, AND MALTOSE.—(Continued.)

[Expressed in Milligrams.]

Cuprous oxid (Cu_2O)	Copper (Cu)	Dextrose (d -glucose)	Invert sugar	Invert sugar and sucrose		Lactose $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$	Maltose $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$	Cuprous oxid (Cu_2O)
				0.4 gram total sugar	2 grams total sugar			
194	172.3	86.2	89.2	87.6	81.4	131.3	159.7	194
195	173.2	86.7	89.6	88.0	81.9	132.0	160.5	195
196	174.1	87.1	90.1	88.5	82.4	132.7	161.4	196
197	175.0	87.6	90.6	89.0	82.9	133.4	162.2	197
198	175.9	88.1	91.1	89.5	83.4	134.1	163.0	198
199	176.8	88.5	91.6	90.0	83.9	134.8	163.9	199
200	177.7	89.0	92.0	90.5	84.4	135.4	164.7	200
201	178.5	89.5	92.5	91.0	84.8	136.1	165.5	201
202	179.4	89.9	93.0	91.4	85.3	136.8	166.4	202
203	180.3	90.4	93.5	91.9	85.8	137.5	167.2	203
204	181.2	90.9	94.0	92.4	86.3	138.2	168.0	204
205	182.1	91.4	94.5	92.9	86.8	138.9	168.9	205
206	183.0	91.8	94.9	93.4	87.3	139.6	169.7	206
207	183.9	92.3	95.4	93.9	87.8	140.3	170.5	207
208	184.8	92.8	95.9	94.4	88.3	141.0	171.4	208
209	185.6	93.2	96.4	94.9	88.8	141.7	172.2	209
210	186.5	93.7	96.9	95.4	89.2	142.3	173.0	210
211	187.4	94.2	97.4	95.8	89.7	143.0	173.8	211
212	188.3	94.6	97.8	96.3	90.2	143.7	174.7	212
213	189.2	95.1	98.3	96.8	90.7	144.4	175.5	213
214	190.1	95.6	98.8	97.3	91.2	145.1	176.4	214
215	191.0	96.1	99.3	97.8	91.7	145.8	177.2	215
216	191.9	96.5	99.8	98.3	92.2	146.5	178.0	216
217	192.8	97.0	100.3	98.8	92.7	147.2	178.9	217
218	193.6	97.5	100.8	99.3	93.2	147.9	179.7	218
219	194.5	98.0	101.2	99.8	93.7	148.6	180.5	219
220	195.4	98.4	101.7	100.3	94.2	149.3	181.4	220
221	196.3	98.9	102.2	100.8	94.7	150.0	182.2	221
222	197.2	99.4	102.7	101.2	95.1	150.7	183.0	222
223	198.1	99.9	103.2	101.7	95.6	151.3	183.9	223
224	199.0	100.3	103.7	102.2	96.1	152.0	184.7	224
225	199.9	100.8	104.2	102.7	96.6	152.7	185.5	225

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR
IN THE PRESENCE OF SUCROSE (0.4 GRAM AND 2 GRAMS TOTAL
SUGAR), LACTOSE, AND MALTOSE.—(Continued.)
[Expressed in Milligrams.]

Cuprous oxid (Cu_2O)	Copper (Cu)	Dextrose (<i>d</i> -glucose)	Invert sugar	Invert sugar and sucrose		Lactose $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$	Maltose $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$	Cuprous oxid (Cu_2O)
				0.4 gram total sugar	2 grams total sugar			
226	200.7	101.3	104.6	103.2	97.1	153.4	186.4	226
227	201.6	101.8	105.1	103.7	97.6	154.1	187.2	227
228	202.5	102.2	105.6	104.2	98.1	154.8	188.0	228
229	203.4	102.7	106.1	104.7	98.6	155.5	188.8	229
230	204.3	103.2	106.6	105.2	99.1	156.2	189.7	230
231	205.2	103.7	107.1	105.7	99.6	156.9	190.5	231
232	206.1	104.1	107.6	106.2	100.1	157.6	191.3	232
233	207.0	104.6	108.1	106.7	100.6	158.3	192.2	233
234	207.9	105.1	108.6	107.2	101.1	159.0	193.0	234
235	208.7	105.6	109.1	107.7	101.6	159.7	193.8	235
236	209.6	106.0	109.5	108.2	102.1	160.3	194.7	236
237	210.5	106.5	110.0	108.7	102.6	161.0	195.5	237
238	211.4	107.0	110.5	109.2	103.1	161.7	196.3	238
239	212.3	107.5	111.0	109.6	103.5	162.4	197.2	239
240	213.2	108.0	111.5	110.1	104.0	163.1	198.0	240
241	214.1	108.4	112.0	110.6	104.5	163.8	198.8	241
242	215.0	108.9	112.5	111.1	105.0	164.5	199.7	242
243	215.8	109.4	113.0	111.6	105.5	165.2	200.5	243
244	216.7	109.9	113.5	112.1	106.0	165.9	201.3	244
245	217.6	110.4	114.0	112.6	106.5	166.6	202.2	245
246	218.5	110.8	114.5	113.1	107.0	167.3	203.0	246
247	219.4	111.3	115.0	113.6	107.5	168.0	203.8	247
248	220.3	111.8	115.4	114.1	108.0	168.7	204.7	248
249	221.2	112.3	115.9	114.6	108.5	169.4	205.5	249
250	222.1	112.8	116.4	115.1	109.0	170.1	206.3	250
251	223.0	113.2	116.9	115.6	109.5	170.8	207.2	251
252	223.8	113.7	117.4	116.1	110.0	171.5	208.0	252
253	224.7	114.2	117.9	116.6	110.5	172.1	208.8	253
254	225.6	114.7	118.4	117.1	111.0	172.8	209.7	254
255	226.5	115.2	118.9	117.6	111.5	173.5	210.5	255
256	227.4	115.7	119.4	118.1	112.0	174.2	211.3	256
257	228.3	116.1	119.9	118.6	112.5	174.9	212.2	257

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR
IN THE PRESENCE OF SUCROSE (0.4 GRAM AND 2 GRAMS TOTAL
SUGAR), LACTOSE, AND MALTOSE.—(Continued.)

[Expressed in Milligrams.]

Cuprous oxid (Cu_2O)	Copper (Cu)	Dextrose (α -glucose)	Invert sugar	Invert sugar and sucrose		Lactose $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$	Maltose $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$	Cuprous oxid (Cu_2O)
				0.4 gram total sugar	2 grams total sugar			
258	229.2	116.6	120.4	119.1	113.0	175.6	213.0	258
259	230.1	117.1	120.9	119.6	113.5	176.3	213.8	259
260	231.0	117.6	121.4	120.1	114.0	177.0	214.7	260
261	231.8	118.1	121.9	120.6	114.5	177.7	215.5	261
262	232.7	118.6	122.4	121.1	115.0	178.4	216.3	262
263	233.6	119.0	122.9	121.6	115.5	179.1	217.2	263
264	234.5	119.5	123.4	122.1	116.0	179.8	218.0	264
265	235.4	120.0	123.9	122.6	116.5	180.5	218.8	265
266	236.3	120.5	124.4	123.1	117.0	181.2	219.7	266
267	237.2	121.0	124.9	123.6	117.5	181.9	220.5	267
268	238.1	121.5	125.4	124.1	118.0	182.6	221.3	268
269	238.9	122.0	125.9	124.6	118.5	183.3	222.1	269
270	239.8	122.5	126.4	125.1	119.0	184.0	223.0	270
271	240.7	122.9	126.9	125.6	119.5	184.6	223.8	271
272	241.6	123.4	127.4	126.2	120.0	185.3	224.6	272
273	242.5	123.9	127.9	126.7	120.6	186.0	225.5	273
274	243.4	124.4	128.4	127.2	121.1	186.7	226.3	274
275	244.3	124.9	128.9	127.7	121.6	187.4	227.1	275
276	245.2	125.4	129.4	128.2	122.1	188.1	228.0	276
277	246.1	125.9	129.9	128.7	122.6	188.8	228.8	277
278	246.9	126.4	130.4	129.2	123.1	189.5	229.6	278
279	247.8	126.9	130.9	129.7	123.6	190.2	230.5	279
280	248.7	127.3	131.4	130.2	124.1	190.9	231.3	280
281	249.6	127.8	131.9	130.7	124.6	191.6	232.1	281
282	250.5	128.3	132.4	131.2	125.1	192.3	233.0	282
283	251.4	128.8	132.9	131.7	125.6	193.0	233.8	283
284	252.3	129.3	133.4	132.2	126.1	193.7	234.6	284
285	253.2	129.8	133.9	132.7	126.6	194.4	235.5	285
286	254.0	130.3	134.4	133.2	127.1	195.1	236.3	286
287	254.9	130.8	134.9	133.7	127.6	195.8	237.1	287
288	255.8	131.3	135.4	134.3	128.1	196.5	238.0	288
289	256.7	131.8	135.9	134.8	128.6	197.1	238.8	289

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR
IN THE PRESENCE OF SUCROSE (0.4 GRAM AND 2 GRAMS TOTAL
SUGAR), LACTOSE, AND MALTOSE.—(Continued.)
[Expressed in Milligrams.]

	Cuprous oxid (Cu_2O)	Copper (Cu)	Dextrose (<i>d</i> -glucose)	Invert sugar	Invert sugar and sucrose		Lactose $\text{C}_{12}\text{H}_{22}\text{O}_{11} - \text{H}_2\text{O}$	Maltose $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$	Cuprous oxid (Cu_2O)
					0.4 gram total sugar	2 grams total sugar			
290	257.6	132.3	136.4	135.3	129.2	197.8	239.6	290	
291	258.5	132.7	136.9	135.8	129.7	198.5	240.5	291	
292	259.4	133.2	137.4	136.3	130.2	199.2	241.3	292	
293	260.3	133.7	137.9	136.8	130.7	199.9	242.1	293	
294	261.2	134.2	138.4	137.3	131.2	200.6	242.9	294	
295	262.0	134.7	138.9	137.8	131.7	201.3	243.8	295	
296	262.9	135.2	139.4	138.3	132.2	202.0	244.6	296	
297	263.8	135.7	140.0	138.8	132.7	202.7	245.4	297	
298	264.7	136.2	140.5	139.4	133.2	203.4	246.3	298	
299	265.6	136.7	141.0	139.9	133.7	204.1	247.1	299	
300	266.5	137.2	141.5	140.4	134.2	204.8	247.9	300	
301	267.4	137.7	142.0	140.9	134.8	205.5	248.8	301	
302	268.3	138.2	142.5	141.4	135.3	206.2	249.6	302	
303	269.1	138.7	143.0	141.9	135.8	206.9	250.4	303	
304	270.0	139.2	143.5	142.4	136.3	207.6	251.3	304	
305	270.9	139.7	144.0	142.9	136.8	208.3	252.1	305	
306	271.8	140.2	144.5	143.4	137.3	209.0	252.9	306	
307	272.7	140.7	145.0	144.0	137.8	209.7	253.8	307	
308	273.6	141.2	145.5	144.5	138.3	210.4	254.6	308	
309	274.5	141.7	146.1	145.0	138.8	211.1	255.4	309	
310	275.4	142.2	146.6	145.5	139.4	211.8	256.3	310	
311	276.3	142.7	147.1	146.0	139.9	212.5	257.1	311	
312	277.1	143.2	147.6	146.5	140.4	213.2	257.9	312	
313	278.0	143.7	148.1	147.0	140.9	213.9	258.8	313	
314	278.9	144.2	148.6	147.6	141.4	214.6	259.6	314	
315	279.8	144.7	149.1	148.1	141.9	215.3	260.4	315	
316	280.7	145.2	149.6	148.6	142.4	216.0	261.2	316	
317	281.6	145.7	150.1	149.1	143.0	216.6	262.1	317	
318	282.5	146.2	150.7	149.6	143.5	217.3	262.9	318	
319	283.4	146.7	151.2	150.1	144.0	218.0	263.7	319	
320	284.2	147.2	151.7	150.7	144.5	218.7	264.6	320	
321	285.1	147.7	152.2	151.2	145.0	219.4	265.4	321	

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR
IN THE PRESENCE OF SUCROSE (0.4 GRAM AND 2 GRAMS TOTAL
SUGAR), LACTOSE, AND MALTOSE.—(Continued.)
[Expressed in Milligrams.]

Cuprous oxid (Cu_2O)	Copper (Cu)	Dextrose (d -glucose)	Invert sugar	Invert sugar and sucrose		Lactose $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$	Maltose $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$	Cuprous oxid (Cu_2O)
				0.4 gram total sugar	2 grams total sugar			
322	286.0	148.2	152.7	151.7	145.5	220.1	266.2	322
323	286.9	148.7	153.2	152.2	146.0	220.8	267.1	323
324	287.8	149.2	153.7	152.7	146.6	221.5	267.9	324
325	288.7	149.7	154.3	153.2	147.1	222.2	268.7	325
326	289.6	150.2	154.8	153.8	147.6	222.9	269.6	326
327	290.5	150.7	155.3	154.3	148.1	223.6	270.4	327
328	291.4	151.2	155.8	154.8	148.6	224.3	271.2	328
329	292.2	151.7	156.3	155.3	149.1	225.0	272.1	329
330	293.1	152.2	156.8	155.8	149.7	225.7	272.9	330
331	294.0	152.7	157.3	156.4	150.2	226.4	273.7	331
332	294.9	153.2	157.9	156.9	150.7	227.1	274.6	332
333	295.8	153.7	158.4	157.4	151.2	227.8	275.4	333
334	296.7	154.2	158.9	157.9	151.7	228.5	276.2	334
335	297.6	154.7	159.4	158.4	152.3	229.2	277.0	335
336	298.5	155.2	159.9	159.0	152.8	229.9	277.9	336
337	299.3	155.8	160.5	159.5	153.3	230.6	278.7	337
338	300.2	156.3	161.0	160.0	153.8	231.3	279.5	338
339	301.1	156.8	161.5	160.5	154.3	232.0	280.4	339
340	302.0	157.3	162.0	161.0	154.8	232.7	281.2	340
341	302.9	157.8	162.5	161.6	155.4	233.4	282.0	341
342	303.8	158.3	163.1	162.1	155.9	234.1	282.9	342
343	304.7	158.8	163.6	162.6	156.4	234.8	283.7	343
344	305.6	159.3	164.1	163.1	156.9	235.5	284.5	344
345	306.5	159.8	164.6	163.7	157.5	236.2	285.4	345
346	307.3	160.3	165.1	164.2	158.0	236.9	286.2	346
347	308.2	160.8	165.7	164.7	158.5	237.6	287.0	347
348	309.1	161.4	166.2	165.2	159.0	238.3	287.9	348
349	310.0	161.9	166.7	165.7	159.5	239.0	288.7	349
350	310.9	162.4	167.2	166.3	160.1	239.7	289.5	350
351	311.8	162.9	167.7	166.8	160.6	240.4	290.4	351
352	312.7	163.4	168.3	167.3	161.1	241.1	291.2	352
353	313.6	163.9	168.8	167.8	161.6	241.8	292.0	353
354	314.4	164.4	169.3	168.4	162.2	242.5	292.8	354

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR
IN THE PRESENCE OF SUCROSE (0.4 GRAM AND 2 GRAMS TOTAL
SUGAR), LACTOSE, AND MALTOSE.—(Continued.)
[Expressed in Milligrams.]

Cuprous oxid. (Cu_2O)	Copper (Cu)	Dextrose (d -glucose)	Invert sugar	Invert sugar and sucrose		Lactose $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$	Maltose $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$	Cuprous oxid. (Cu_2O)
				0.4 gram total sugar	2 grams total sugar			
355	315.3	164.9	169.8	168.9	162.7	243.2	293.7	355
356	316.2	165.4	170.4	169.4	163.2	243.9	294.5	356
357	317.1	166.0	170.9	170.0	163.7	244.6	295.3	357
358	318.0	166.5	171.4	170.5	164.3	245.3	296.2	358
359	318.9	167.0	171.9	171.0	164.8	246.0	297.0	359
360	319.8	167.5	172.5	171.5	165.3	246.7	297.8	360
361	320.7	168.0	173.0	172.1	165.8	247.4	298.7	361
362	321.6	168.5	173.5	172.6	166.4	248.1	299.5	362
363	322.4	169.0	174.0	173.1	166.9	248.8	300.3	363
364	323.3	169.6	174.6	173.7	167.4	249.5	301.2	364
365	324.2	170.1	175.1	174.2	167.9	250.2	302.0	365
366	325.1	170.6	175.6	174.7	168.5	250.9	302.8	366
367	326.0	171.1	176.1	175.2	169.0	251.6	303.6	367
368	326.9	171.6	176.7	175.8	169.5	252.3	304.5	368
369	327.8	172.1	177.2	176.3	170.0	253.0	305.3	369
370	328.7	172.7	177.7	176.8	170.6	253.7	306.1	370
371	329.5	173.2	178.3	177.4	171.1	254.4	307.0	371
372	330.4	173.7	178.8	177.9	171.6	255.1	307.8	372
373	331.3	174.2	179.3	178.4	172.2	255.8	308.6	373
374	332.2	174.7	179.8	179.0	172.7	256.5	309.5	374
375	333.1	175.3	180.4	179.5	173.2	257.2	310.3	375
376	334.0	175.8	180.9	180.0	173.7	257.9	311.1	376
377	334.9	176.3	181.4	180.6	174.3	258.6	312.0	377
378	335.8	176.8	182.0	181.1	174.8	259.3	312.8	378
379	336.7	177.3	182.5	181.6	175.3	260.0	313.6	379
380	337.5	177.9	183.0	182.1	175.9	260.7	314.5	380
381	338.4	178.4	183.6	182.7	176.4	261.4	315.3	381
382	339.3	178.9	184.1	183.2	176.9	262.1	316.1	382
383	340.2	179.4	184.6	183.8	177.5	262.8	316.9	383
384	341.1	180.0	185.2	184.3	178.0	263.5	317.8	384
385	342.0	180.5	185.7	184.8	178.5	264.2	318.6	385
386	342.9	181.0	186.2	185.4	179.1	264.9	319.4	386
387	343.8	181.5	186.8	185.9	179.6	265.6	320.3	387

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR IN THE PRESENCE OF SUCROSE (0.4 GRAM AND 2 GRAMS TOTAL SUGAR), LACTOSE AND MALTOSE.—(*Continued.*)
[Expressed in Milligrams.]

Cuprous oxid (Cu ₂ O)	Copper (Cu)	Dextrose (d-glucose)	Invert sugar	Invert sugar and sucrose		Lactose C ₁₂ H ₂₂ O ₁₁ + H ₂ O	Maltose C ₁₂ H ₂₂ O ₁₁ + H ₂ O	Cuprous oxid (Cu ₂ O)
				0.4 gram total sugar	2 grams total sugar			
388	344.6	182.0	187.3	186.4	180.1	266.3	321.1	388
389	345.5	182.6	187.8	187.0	180.6	267.0	321.9	389
390	346.4	183.1	188.4	187.5	181.2	267.7	322.8	390
391	347.3	183.6	188.9	188.0	181.7	268.4	323.6	391
392	348.2	184.1	189.4	188.6	182.3	269.1	324.4	392
393	349.1	184.7	190.0	189.1	182.8	269.8	325.2	393
394	350.0	185.2	190.5	189.7	183.3	270.5	326.1	394
395	350.9	185.7	191.0	190.2	183.9	271.2	326.9	395
396	351.8	186.2	191.6	190.7	184.4	271.9	327.7	396
397	352.6	186.8	192.1	191.3	184.9	272.6	328.6	397
398	353.5	187.3	192.7	191.8	185.5	273.3	329.4	398
399	354.4	187.8	193.2	192.3	186.0	274.0	330.2	399
400	355.3	188.4	193.7	192.9	186.5	274.7	331.1	400
401	356.2	188.9	194.3	193.4	187.1	275.4	331.9	401
402	357.1	189.4	194.8	194.0	187.6	276.1	332.7	402
403	358.0	189.9	195.4	194.5	188.1	276.8	333.6	403
404	358.9	190.5	195.9	195.0	188.7	277.5	334.4	404
405	359.7	191.0	196.4	195.6	189.2	278.2	335.2	405
406	360.6	191.5	197.0	196.1	189.8	278.9	336.0	406
407	361.5	192.1	197.5	196.7	190.3	279.6	336.9	407
408	362.4	192.6	198.1	197.2	190.8	280.3	337.7	408
409	363.3	193.1	198.6	197.7	191.4	281.0	338.5	409
410	364.2	193.7	199.1	198.3	191.9	281.7	339.4	410
411	365.1	194.2	199.7	198.8	192.5	282.4	340.2	411
412	366.0	194.7	200.2	199.4	193.0	283.2	341.0	412
413	366.9	195.2	200.8	199.9	193.5	283.9	341.9	413
414	367.7	195.8	201.3	200.5	194.1	284.6	342.7	414
415	368.6	196.3	201.8	201.0	194.6	285.3	343.5	415
416	369.5	196.8	202.4	201.6	195.2	286.0	344.4	416
417	370.4	197.4	202.9	202.1	195.7	286.7	345.2	417
418	371.3	197.9	203.5	202.6	196.2	287.4	346.0	418
419	372.2	198.4	204.0	203.2	196.8	288.1	346.8	419
420	373.1	199.0	204.6	203.7	197.3	288.8	347.7	420

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR IN THE PRESENCE OF SUCROSE (0.4 GRAM AND 2 GRAMS TOTAL SUGAR), LACTOSE AND MALTOSE.—(Continued.)
[Expressed in Milligrams.]

Cuprous oxid (Cu_2O)	Copper (Cu)	Dextrose (d-glucose)	Invert sugar	Invert sugar and sucrose		Lactose $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$	Maltose $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$	Cuprous oxid (Cu_2O)
				0.4 gram total sugar	2 grams total sugar			
421	374.0	199.5	205.1	204.3	197.9	289.5	348.5	421
422	374.8	200.1	205.7	204.8	198.4	290.2	349.3	422
423	375.7	200.6	206.2	205.4	198.8	290.9	350.2	423
424	376.6	201.1	206.7	205.9	199.5	291.6	351.0	424
425	377.5	201.7	207.3	206.5	200.0	292.3	351.8	425
426	378.4	202.2	207.8	207.0	200.6	293.0	352.7	426
427	379.3	202.8	208.4	207.6	201.1	293.7	353.5	427
428	380.2	203.3	208.9	208.1	201.7	294.4	354.3	428
429	381.1	203.8	209.5	208.7	202.2	295.1	355.1	429
430	382.0	204.4	210.0	209.2	202.7	295.8	356.0	430
431	382.8	204.9	210.6	209.8	203.3	296.5	356.8	431
432	383.7	205.5	211.1	210.3	203.8	297.2	357.6	432
433	384.6	206.0	211.7	210.9	204.4	297.9	358.5	433
434	385.5	206.5	212.2	211.4	204.9	298.6	359.3	434
435	386.4	207.1	212.8	212.0	205.5	299.3	360.1	435
436	387.3	207.6	213.3	212.5	206.0	300.0	361.0	436
437	388.2	208.2	213.9	213.1	206.6	300.7	361.8	437
438	389.1	208.7	214.4	213.6	207.1	301.4	362.6	438
439	390.0	209.2	215.0	214.2	207.7	302.1	363.4	439
440	390.8	209.8	215.5	214.7	208.2	302.8	364.3	440
441	391.7	210.3	216.1	215.3	208.8	303.5	365.1	441
442	392.6	210.9	216.6	215.8	209.3	304.2	365.9	442
443	393.5	211.4	217.2	216.4	209.9	304.9	366.8	443
444	394.4	212.0	217.8	216.9	210.4	305.6	367.6	444
445	395.3	212.5	218.3	217.5	211.0	306.3	368.4	445
446	396.2	213.1	218.9	218.0	211.5	307.0	369.3	446
447	397.1	213.6	219.4	218.6	212.1	307.7	370.1	447
448	397.9	214.1	220.0	219.1	212.6	308.4	370.9	448
449	398.8	214.7	220.5	219.7	213.2	309.1	371.7	449
450	399.7	215.2	221.1	220.2	213.7	309.9	372.6	450
451	400.6	215.8	221.6	220.8	214.3	310.6	373.4	451
452	401.5	216.3	222.2	221.4	214.8	311.3	374.2	452
453	402.4	216.9	222.8	221.9	215.4	312.0	375.1	453
454	403.3	217.4	223.3	222.5	215.9	312.7	375.9	454
455	404.2	218.0	223.9	223.0	216.5	313.4	376.7	455

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR IN THE PRESENCE OF SUCROSE (0.4 GRAM AND 2 GRAMS TOTAL SUGAR), LACTOSE AND MALTOSE. — (Continued.)
[Expressed in Milligrams.]

Cuprous oxid (Cu_2O)	Copper (Cu)	Dextrose (α -glucose)	Invert sugar	Invert sugar and sucrose		Lactose $\text{C}_{12}\text{H}_{22}\text{O}_{11} - \text{H}_2\text{O}$	Maltose $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$	Cuprous oxid (Cu_2O)
				0.4 gram total sugar	2 grams total sugar			
456	405.1	218.5	224.4	223.6	217.0	314.1	377.6	456
457	405.9	219.1	225.0	224.1	217.6	314.8	378.4	457
458	406.8	219.6	225.5	224.7	218.1	315.5	379.2	458
459	407.7	220.2	226.1	225.3	218.7	316.2	380.0	459
460	408.6	220.7	226.7	225.8	219.2	316.9	380.9	460
461	409.5	221.3	227.2	226.4	219.8	317.6	381.7	461
462	410.4	221.8	227.8	226.9	220.3	318.3	382.5	462
463	411.3	222.4	228.3	227.5	220.9	319.0	383.4	463
464	412.2	222.9	228.9	228.1	221.4	319.7	384.2	464
465	413.0	223.5	229.5	228.6	222.0	320.4	385.0	465
466	413.9	224.0	230.0	229.2	222.5	321.1	385.9	466
467	414.8	224.6	230.6	229.7	223.1	321.8	386.7	467
468	415.7	225.1	231.2	230.3	223.7	322.5	387.5	468
469	416.6	225.7	231.7	230.9	224.2	323.2	388.3	469
470	417.5	226.2	232.3	231.4	224.8	323.9	389.2	470
471	418.4	226.8	232.8	232.0	225.3	324.6	390.0	471
472	419.3	227.4	233.4	232.5	225.9	325.3	390.8	472
473	420.2	227.9	234.0	233.1	226.4	326.0	391.7	473
474	421.0	228.5	234.5	233.7	227.0	326.8	392.5	474
475	421.9	229.0	235.1	234.2	227.6	327.5	393.3	475
476	422.8	229.6	235.7	234.8	228.1	328.2	394.2	476
477	423.7	230.1	236.2	235.4	228.7	328.9	395.0	477
478	424.6	230.7	236.8	235.9	229.2	329.6	395.8	478
479	425.5	231.3	237.4	236.5	229.8	330.3	396.6	479
480	426.4	231.8	237.9	237.1	230.3	331.3	397.5	480
481	427.3	232.4	238.5	237.6	230.9	331.7	398.3	481
482	428.1	232.9	239.1	238.2	231.5	332.4	399.1	482
483	429.0	233.5	239.6	238.8	232.0	333.1	400.0	483
484	429.9	234.1	240.2	239.3	232.6	333.8	400.8	484
485	430.8	234.6	240.8	239.9	233.2	334.5	401.6	485
486	431.7	235.2	241.4	240.5	233.7	335.2	402.4	486
487	432.6	235.7	241.9	241.0	234.3	335.9	403.3	487
488	433.5	236.3	242.5	241.6	234.8	336.6	404.1	488
489	434.4	236.9	243.1	242.2	235.4	337.3	404.9	489
490	435.3	237.4	243.6	242.7	236.0	338.0	405.8	490

187. Methods of Determining the Copper Contained in the Precipitate of Cuprous Oxid.—The following methods have been recommended for this determination:

(1) The solution of the cuprous oxid in sulfuric acid and potassium chlorate, the addition of ammonia and titration. (2) The reduction of ferric sulfate to ferrous sulfate by the cuprous oxid and the titration of this solution by permanganate. (3) Low's method or the titration with sodium thiosulfate after solution of the cuprous oxid. (4) The reduction of the cuprous oxid to metallic copper in a stream of hydrogen. (5) The direct weighing of the cuprous oxid has been mentioned in Munson and Walker's method. (6) Electrolytic deposition of the copper after solution of the cuprous oxid. (7) Collection of the precipitated cuprous oxid on a Munroe⁹⁴ crucible and heating to redness and weighing as cupric oxid. The Munroe crucible is a platinum gooch crucible with a filtering medium of platinum sponge. Zerban and Naquin⁹⁵ found this to give good results and to be quicker than the ordinary porcelain crucible. Walker⁹⁶ took exception to some of their views on the subject.

188. Discussion of the Above Methods.—The electrolytic method and the reduction method are seldom used in this country now. The former was the one almost generally used until some six years ago. But as the plating has to be done carefully and under strict conditions so as to obtain a solid one and there is a chance of not obtaining all the copper, it has been superseded by other methods. The latter method is used abroad almost exclusively where gravimetric sugar methods are used, but it finds little use in this country. The volumetric methods using ammonia and also the ferric sulfate process are not extensively used, although the latter method gives good results and is fairly quick. The direct weighing of cuprous oxid has come into use quite extensively since the work of Knight⁹⁷ and Munson⁹⁸

⁹⁴ *Journal of the American Chemical Society*, 1909, **31**: 456.

⁹⁵ *Journal of the American Chemical Society*, 1908, **30**: 1456.

⁹⁶ *International Sugar Journal* 1908, **10**: 441.

⁹⁷ *Bureau of Chemistry Bulletin* 62, 1900: 111.

⁹⁸ *Bureau of Chemistry Bulletin* 73, 1902: 64.

has shown its excellence. It is easy of manipulation and when the crucibles are carefully prepared with good asbestos, the results are accurate for pure sugar solutions. But if the sugar solution under examination contains much organic matter, especially of albuminoid nature as shown by Browne⁹⁹ and later by Bryan¹ the precipitated cuprous oxid will carry a portion of this down and thereby increase the percentage of apparent reducing sugars present. To remedy this, the heating of the crucibles after filtering the cuprous oxid, in a muffle to red heat and the consequent oxidation of the cuprous oxid to cupric oxid was tried. While burning out the organic matter by this method and a complete oxidation of the cuprous oxid are secured, yet there is a chance for reduction of some of the cuprous oxide to copper and worse still, a shortening of the life of the asbestos mat. For with this method of weighing as cuprous oxid, the crucibles are used over and over again, the red oxid being dissolved out with hot nitric acid; and the older the crucible within limits, the better it is for the work. The weighing as cuprous oxid also introduces another error which cannot be corrected by oxidizing to the cupric state. In treating sugar solutions containing much mineral matter by this method, some of the mineral substance is precipitated along with the cuprous oxid in the very alkaline solution. In such cases, the apparent reducing sugar percentage is too high. The results obtained from the use of Munroe crucibles would be in error on this account with such material. It is better therefore to determine the copper in the cuprous oxid in some other way. Low's method, titration with sodium thio-sulfate after solution of the cuprous oxid, has been found by the Association of Official Agricultural Chemists to give good results and though long in procedure, such results can be relied upon.²

⁹⁹ Bureau of Chemistry Bulletin 105, 1907: 121. Bureau of Chemistry Bulletin 116, 1908: 73.

¹ Bureau of Chemistry Bulletin 122, 1909: 180 and Bulletin 132, 1910: 180.

² Journal of the American Chemical Society 1902, 24: 1082. Bureau of Chemistry Bulletin 107 revised: 241.

189. Preparation of Asbestos for Mats.—This is an important point especially if the cuprous oxid is to be directly weighed and in any case, the material should be carefully prepared. The Association of Official Agricultural Chemists, recommend the following procedure for cleaning the asbestos and filling the crucibles.³ The asbestos should be the amphibole variety and on scraping to a fluffy mass must be free of grit and not harsh to the feel. It is first digested two or three days in hydrochloric acid 1:3. Wash the digested fiber free from acid and digest for a similar period with soda solution, after which treat for a few hours with hot alkaline copper tartrate solution of the strength employed in sugar determinations. Then wash the asbestos free from alkali, finally digest with nitric acid for several hours, and after washing free from acid shake with water for use. In preparing the gooch crucible load it with a film of asbestos one-fourth inch thick, wash this thoroughly with water to remove fine particles of asbestos; finally wash with alcohol and ether, dry for 30 minutes at 100° C., cool in a desiccator and weigh. The latter weighing is for use in the direct weighing of the cuprous oxid. It is best to dissolve the cuprous oxid with nitric acid each time after weighing and use the same felts over and over again, since they improve with use.

190. Errors Coming from Manipulation.—In all gravimetric work, as well as volumetric, there is an error introduced through the spontaneous precipitation of cuprous oxid. In volumetric work this can hardly be corrected but in gravimetric work, a blank can be run to determine this. This comes about by the fact that when alkaline copper tartrate is boiled, a slight precipitation of cuprous oxid takes place. When using inferior chemicals or impure water in preparing solutions this may amount to a rather large figure. All Fehling solutions should be tested by boiling to see the extent of this error. When using the weight method for cuprous oxid there is an error due to the solubility of the asbestos in the solution. As the mats are used over and

³ Journal of the American Chemical Society, 1906, 28: 666, 1907, 29: 541. Bureau of Chemistry, Bulletin 107 revised: 241.

over again this solubility reaches a minimum point and in some cases amounts to nothing. The error due to these two causes in Munson and Walker's work varied between -1.3 to $+2.0$ milligrams in weight. For most work, however, these errors can be neglected. However, for very accurate work and especially in weighing small quantities, it should be considered and an account made of it.

Mention has been made of the importance of following the method of manipulation to the exact letter in order to obtain results that are correct. For instance the method of heating the solution for reduction is very important as the following table compiled by Munson shows.⁴

REDUCING VALUES FOR DEXTROSE AND INVERT SUGAR OBTAINED BY THREE DIFFERENT METHODS OF HEATING.

Sugar	Glucose			Invert Sugar		
	Copper boiling 2 minutes	Copper by heating in water bath 12 minutes	Copper by heating in water bath 20 minutes	Copper by boiling 2 minutes	Copper by heating in water bath 12 minutes	Copper by heating in water bath 20 minutes
	a Mgs.	b Mgs.	c Mgs.	d Mgs.	b Mgs.	c Mgs.
50	98.2	103.0	111.4	96.0	97.5	105.6
75	147.2	154.3	163.2	142.8	146.8	155.6
100	195.0	202.7	212.5	188.8	194.1	203.4
125	242.0	249.6	259.2	233.2	240.0	249.0 ¹
150	288.2	293.4	303.3	276.8	283.2	292.3
175	333.4	335.0	345.0	318.8	325.1	333.7
200	377.5	374.5	384.0	360.4	365.0	372.7

a Figures from Allihn's table for dextrose.

b " " Brown, Morris and Millar,
Journal Chemical Society Transactions, 1897 : 275.

c " " Kjeldahl tables.

d " " Meissl's table for invert sugar.

By changing the method of heating and also the time the results are very largely influenced especially is this so in the lesser quantity of the reducing sugar. In the following table, taken also from Munson, the results of just bringing to a boil over a free flame and for varying lengths of time are given.⁵

⁴ Bureau of Chemistry, Bulletin 73, 1903 : 61.

⁵ Bureau of Chemistry, Bulletin 73, 1903 : 62.

COPPER REDUCED UPON BOILING OVER FREE FLAME FOR
VARYING PERIODS.

Sugar	Time of heating	Copper precipitated	Increase of copper
250 mgs. glucose	Brought to boil,	437.2 mgs.	
250 mgs. glucose	Boiled 2 minutes,	439.5 mgs.	2.3
125 mgs. glucose	Brought to boil,	227.9 mgs.	
125 mgs. glucose	Boiled 2 minutes,	229.8 mgs.	1.9
185.9 mgs. invert sugar....	Brought to boil,	245.1 mgs.	
185.9 mgs. invert sugar....	Boiled 1 minute,	346.8 mgs.	1.7
185.9 mgs. invert sugar....	Boiled 2 minutes,	347.6 mgs.	0.8
185.9 mgs. invert sugar....	Boiled 3 minutes,	348.7 mgs.	1.1
185.9 mgs. invert sugar....	Boiled 4 minutes,	350.4 mgs.	1.7

Increase of time gives more copper. The tables belonging with the particular method are based on prescribed length of time and manner of heating so any deviation from these rules will render the results of doubtful value.

The amount of surface of liquid exposed during the reduction influences the amount of copper reduced. The following table from Kjeldahl⁶ shows this.

EFFECT OF AMOUNT OF SURFACE EXPOSED TO AIR UPON THE
COPPER REDUCED.

Sugar milligrams	Surface exposed to air square centimeters	Copper reduced milligrams	Difference milligrams
60	a	126.3	
60	2	126	0.3
60	17	123.9	2.4
60	21	121.5	4.8
60	65	114.8	11.5
60	186	106.6	19.7

a None; reduction in hydrogen.

As the surface exposure increases the amount of cuprous oxid decreases. In other words, the oxidation of the cuprous oxid by exposure to air is very marked. An exposure of 186 square centimeters is hardly probable in analytical work as that is an extreme; however, exposures of 15 to 65 square centimeters are of frequent occurrence. Munson recommends a beaker of about 75 centimeters diameter for work which would give an exposure of 44.17 square centimeters.

⁶ Comptes Rendus des Travaux du Laboratoire de Carlsberg, 1895, 4: 5. Bureau of Chemistry, Bulletin 73, 1903: 63.

191. Clarification for Reducing Sugar Determination.—In raw sugar analysis, it is the practice of the sugar chemists of all countries except Great Britain to clarify the solutions. This clarifying agent should be neutral lead acetate and never basic lead acetate. When lead has been used, it must be removed before the sugar determination can be made. This is accomplished by any of the usual reagents for the precipitation of soluble lead salts preferably sodium carbonate. When determining the reducing sugars after inversion, the acidity must be neutralized, also if working with an acid solution of any kind this acidity should be neutralized before making a reduction. For the same reason, if a very alkaline solution is to be analyzed, this alkalinity should be reduced to near neutrality before the determination is made.

The presence of alcohol in a solution for reductions has been thought to be harmful. Methods for wine analysis have stated that the solution should be boiled after neutralization to remove the alcohol. West⁷ has found that a strong solution of alcohol failed to reduce Fehling solution and that the reducing sugar obtained by using the wine direct and by boiling off the alcohol were comparable. However, if much alcohol is present, evaporation will change the volume of the solution during reduction and will in that way influence the amount of cuprous oxid obtained. In concentrating solutions for reducing sugar determinations, acidity and alkalinity should be neutralized as both tend to decompose some of the sugars.

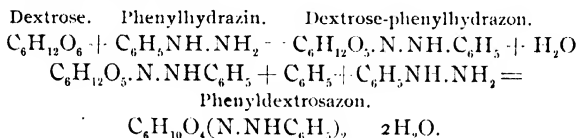
Sodium hydroxid can be used to neutralize acidity provided the solution is left slightly acid. Sodium carbonate is better and dry calcium carbonate is preferable in many cases.

192. Precipitation of Sugars with Phenylhydrazin.—The combination of phenylhydrazin with aldehyds and ketones was first studied by Fischer, and the near relationship of these bodies to sugar soon lead to the investigation of the compounds formed thereby with this reagent.⁸ Reducing sugars form with phenyl-

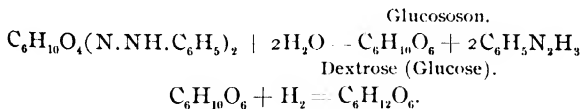
⁷ Bureau of Chemistry, Bulletin 122 : 15.

⁸ *Berichte der deutschen chemischen Gesellschaft*, 1883, 16 : 661.

hydrazin two bodies (1) in the cold phenylhydrazons (2) by heating phenylosazons a few of the former are crystalline and separate out as for instance mannose, but the greater portion are soluble. The osazons for the most part are crystalline and are insoluble or only partially soluble, while some like maltose osazon is soluble in hot water but insoluble in cold. They have melting points which vary a little so identification might be made by this means. The reaction which takes place is represented by the following formulas:



The osazones are precipitated in the following way: The reducing sugar, in about 10 per cent. solution is treated with an excess of phenylhydrazin and acetic acid and immersed in a beaker in a rapidly boiling water bath for an hour or so. In a short time a separation occurs and the yellow precipitate formed is collected on filter paper and may be purified by crystallization from a dilute solution of pyridine⁹ and be dried and weighed if desired. The sugar can be recovered from the osazon by decomposing it with strong hydrochloric acid by means of which the phenylhydrazin is displaced and a body, *osone*, is formed, which by treatment with zinc dust and acetic acid, is reduced to the original sugar.¹⁰ The reactions which take place are represented by the following equations:



But to recover the sugar from the hydrazon, benzaldehyd has been found better and still later, formaldehyd has been used. Using the latter, the hydrazon is dissolved in dilute formyldehyd and heated to the temperature of the water bath. The excess of

⁹ Armstrong, The Simple Carbohydrates and the Glucosides, 1910: 22.

¹⁰ Berichte der deutschen chemischen Gesellschaft, 1869, 22: 95.

formaldehyde is removed from the solution and the pure sugar solution concentrated in vacuum.

For the complete precipitation of dextrose as osazon Lintner and Kröber show that the solution of dextrose should not contain more than one gram in 100 cubic centimeters. Twenty cubic centimeters containing 0.2 gram dextrose should be used for the precipitation.¹¹ To this solution should be added one gram of phenylhydrazin and one gram of 50 per cent. acetic acid. The solution is then to be warmed for about two hours and the precipitate washed with from 60 to 80 cubic centimeters of hot water and dried for three hours at 105°. One part of the osazon is equivalent to one part of dextrose when maltose and dextrin are absent. When these are present the proportion is one part of osazone to 1.04 of dextrose. Where levulose is precipitated instead of dextrose 1.43 parts of the osazon are equal to one part of the sugar.

Sucrose is scarcely at all precipitated as osazon until inverted.

After inversion and precipitation as above, 1.33 parts osazon are equal to one part of sucrose.

Maquenne has studied the action of phenylhydrazin on sugars and considers that this reaction offers the only known means of precipitating these bodies from solutions where they are found mixed with other substances.¹² The osazons, which are thus obtained, are usually very slightly soluble in the ordinary reagents, for which reason it is easy to obtain them pure when there is at the disposition of the analyst a sufficient quantity of the material. But if the sugar to be studied is rare and if it is mixed with other reducing bodies, the task is more delicate. It is easy then to confound several osazons which have almost identical points of fusion; for example, gilucosazon with galactosazon. Finally, it becomes impossible by the employment of phenylhydrazin to distinguish glucose, or mannose from levulose alone or mixed with its isomers. Indeed, these three sugars give, with phenylhydrazin the same phenylosazon which melts at about

¹¹ *Chemisches Centralblatt*, 1895, 2: 66.

¹² *Comptes rendus des seances de l'Académie des sciences*, 1891, 112: 799.

208°. The melting point may vary considerably depending on the manner of heating. On the same compound 213° was found when heating was carried on fast while 195° when heated slower.¹³ It is noticed that the weights of osazons which are precipitated when different sugars are heated for the same time with the same quantity of the phenylhydrazin, vary within extremely wide limits. It is constant for each kind of sugar if the conditions under which the precipitation is made are rigorously the same. There is then, in the weight of osazons produced, a new characteristic of particular value. The following numbers have been obtained by heating for one hour at 100°, one gram of sugar with 100 cubic centimeters of water and five cubic centimeters of a solution containing 40 grams of phenylhydrazin and 40 grams of acetic acid per 100. After cooling the liquid, the osazons are received upon a weighed filter, washed with 100 cubic centimeters of water, dried at 110° and weighed. The weights of osazons obtained are given in the following table:

Character of the sugar	Weight of the osazons gram
Sorbose, crystallized.....	0.82
Levulose ".....	0.70
Xylose ".....	0.40
Glucose, anhydrous.....	0.32
Arabinose, crystallized.....	0.27
Galactose ".....	0.23
Rhamnose ".....	0.15
Lactose ".....	0.11
Maltose ".....	0.11

With solutions twice as dilute as those above, the relative conditions are still more sensible, and the different sugars arrange themselves in the same order, with the exception of levulose, which shows a slight advantage over sorbose and acquires the first rank. From the above determinations, it is shown that levulose and sorbose give vastly greater quantities of osazons, under given conditions, than the other reducing sugars. It would be easy, therefore, to distinguish them by this reaction and to recognize their presence also even in very complex mixtures, where the polarimetric examination alone would furnish only uncertain indications.

¹³ Armstrong, Simple Carbohydrates and Glucosides, 1910 : 23.

It is remarkable that these two sugars are the only ones among the isomers or the homologues of dextrose, actually known, which possess the functions of a ketone. They are not, however, easily confounded, since the glucosazon forms beautiful needles which are ordinarily visible to the naked eye, while the sorbinosazon is still oily and when heated never gives perfectly distinct crystals.

This method also enables us to distinguish between dextrose and galactose, of which the osazon is well crystallized and melts at almost the same temperature as the phenylglucosazon. Finally, it is observed that the reducing sugars give less of osazons than the sugars which are not capable of hydrolysis, and consequently differ in their inversion products. It is specially noticed in this study of the polyglucoses (bioses, trioses), that this new method of employing the phenylhydrazin appears very advantageous. It is sufficient to compare the weights of the osazons to that which is given under the same conditions by a known glucose, in order to have a very certain verification of the probabilities of the result of the chemical or optical examination of the mixture which is under study. All the polyglucoses which have been examined from this point of view give very decided results. The numbers which follow have reference to one gram of sugar completely inverted by dilute sulfuric acid, dissolved in 100 cubic centimeters of water, and treated with two grams of phenylhydrazin, the same quantity of acetic acid, and five grams of crystallized sodium acetate. All these solutions have been compared with the artificial mixtures of the component glucoses, with the same quantities of the same reagents. The following are the results of the examination:

Character of the sugar	Weight of the osazon gram
1 { Sucrose, ordinary (after inversion).....	0.71
1 { Glucose and levulose (.526 g. each)	0.73
2 { Maltose.....	0.55
2 { Glucose (1.052 g.)	0.58
3 { Raffinose, crystallized	0.48
3 { Levulose, glucose and galactose (.333 g. each).....	0.53
4 { Lactose, crystallized..	0.38
4 { Glucose and galactose (.500 g. each)	0.39

It is noticed that the agreement for each sugar and the artificial mixture of the monosaccharids composing the sugar is as satisfactory as possible. Numbers obtained with the products of inversion are always a little low by reason of the destructive action of hydrochloric acid upon levulose. This is, moreover, quite possible when we consider the product has to be heated for a long time with hydrochloric acid in order to secure a complete inversion. Besides phenylhydrazin, there have been used a great many substitution products of this, for instance methylphenyl, benzylphenyl or diphenyl hydrazins. The hydrazons formed with these are often quite insoluble and characteristic of a peculiar sugar. Armstrong has revised the melting points of the hydrazons and osazons and the data ascertained are given in the following table:

MELTING POINTS OF SUGAR HYDRAZONS AND OSAZONS.

Hydrazons	Arabinose	Glucose	Mannose	Galac- tose	Mal- tose	Lac- tose
Phenylhydrazon	151-153°	$\left\{ \begin{array}{l} 115^{\circ}-116^{\circ} \\ 144^{\circ}-146^{\circ} \end{array} \right\}$	186°-188°	158°	—	—
β -Bromophenylhydrazon	150°	147°	208°-210°	168°	—	—
α -Methyl " "	161°	130°	178°	180°	—	—
α -Ethyl " "	153°	—	159°	169°	—	—
α -Amyl " "	120°	128°	134°	116°	—	123°
α -Allyl " "	145°	155°	142°	157°	—	132°
α -Benzoyl " "	170°	165°	165°	154°	—	128°
Diphenyl " "	218°	161°	155°	157°	—	—
β -Naphthylhydrazon	141°	—	157°	167°	176°	203°
Osazons						
Phenylosazon	160°	208°	208°	193°	206°	200°
β -Bromophenylosazon	196-200°	222°	—	—	198°	—
β -Nitrophenyl " "	—	257°	—	—	261°	258°

It is evident from the data cited from the papers of Fischer, Maquenne, and others, that the identification of sugars by this method is not a very difficult analytical process, but so far the quantitative estimation of sugars by this method is not possible.

193. Molecular Weights of Carbohydrates.—In the examination of carbohydrates the determination of the molecular weights is often of the highest analytical value.

The uncertainty in respect of the true molecular weights of the carbohydrates is gradually disappearing by reason of the insight into the composition of these bodies, which recently discovered physical relations have permitted.

Raoult, many years ago,¹¹ proposed a method of determining molecular weights which is particularly applicable to carbohydrates soluble in water.

The principle of Raoult's discovery may be stated as follows: The depression of the freezing-point of a liquid, caused by the presence of a dissolved liquid or solid, is proportionate to the absolute amount of substance dissolved and inversely proportionate to its molecular weight.

The following formulas may be used in computing results:

C = observed depression of freezing-point:

P = weight of anhydrous substance in 100 grams:

$\frac{C}{P} = A$ — depression produced by one gram substance in 100 grams:

K = depression produced by dissolving in 100 cubic centimeters a number of grams of the substance corresponding to its molecular weight:

M = molecular weight:

Then we have, $K = \frac{C}{P} \times M$.

K is a quantity varying with the nature of the solvent but with the same solvent remaining sensibly constant for numerous groups of compounds.

The value of $A \left(\frac{C}{P} \right)$ can be determined by experiment. The molecular weight can therefore be calculated from the formula

$$M = \frac{K}{A}.$$

¹¹ Comptes rendus des séances de l'Académie des Sciences, 1882, 94: 1517.

With organic compounds in water the value of K is almost constant.

Brown and Morris¹⁵ report results of their work in extending Raoult's investigations of the molecular weight of the carbohydrates. The process is carried on as follows:

A solution of the carbohydrate is prepared containing a known weight of the substance in 100 cubic centimeters of water. About 120 cubic centimeters of the solution are introduced into a thin beaker of about 400 capacity. This beaker is closed with a stopper with three holes. Through one of these a glass rod for stirring the solution is inserted. The second perforation carries a delicate thermometer graduated to $0^{\circ}.05$. The temperature is read with a telescope. The beaker is placed in a mixture of ice and brine at a temperature from 2° to 3° below the freezing-point of the solution. The solution is cooled until its temperature is from $0^{\circ}.5$ to 1° below the point of congelation. Through the third aperture in the stopper a small lump of ice taken from a frozen portion of the same solution, is dropped, causing at once the freezing process to begin. The liquid is briskly stirred and as the congelation goes on the temperature rises and finally becomes constant. The reading is then taken. The depression in the freezing-point, controlled by the strength of the solution, should never be more than from 1° to 2° .

The molecular weights may also be determined by the boiling points of their solutions as indicated by the author,¹⁶ Beckmann,¹⁷ Hite, Orndorff and Cameron.¹⁸

The method applied to some of the more important carbohydrates gave the following results:

¹⁵ *Journal of the Chemical Society*, 1888, **53**: 610. (In the formulas for lactose and arabinose read H_{22} and H_{10} respectively).

¹⁶ *American Chemical Journal*, 1889, **11**: 469.

¹⁷ *Chemisches Centralblatt*, 1889, **2**: 278.

¹⁸ *American Chemical Journal*, 1895, **17**: 507, 517.

DEXTROSE.	
Calculated for $C_6H_{12}O_6$.	Found.
$M = 180$	$M = 180.2$
SUCROSE.	
Calculated for $C_{12}H_{22}O_{11}$.	Found.
$M = 342$	$M = 337.5$
INVERT SUGAR (DEXTROSE AND LEVULOSE).	
Calculated for $C_6H_{12}O_6$.	Found.
$M = 180$	$M = 174.3$
MALTOSE.	
Calculated for $C_{12}H_{22}O_{11}$.	Found.
$M = 342$	$M = 322$
LACTOSE.	
Calculated for $C_{12}H_{22}O_{11}$.	Found.
$M = 342$	$M = 345$
ARABINOSE.	
Calculated for $C_5H_{10}O_5$.	Found.
$M = 150$	$M = 150.3$
RAFFINOSE.	
Calculated for $C_{18}H_{32}O_{16}H_2O$.	Found.
$M = 594$	$M = 528$

194. Estimation of Pentosans by Acid Distillation.—The production of furfural and also methylfurfural by distilling carbohydrates with an acid has already been mentioned. Tollens and his associates have shown that with pentose sugars, and carbohydrate bodies yielding them, the production of furfural is quantitative and likewise with methylpentoses and methylpentosans the production of methylfurfural is quantitative.

The production and estimation of furfural have been systematically studied by Krug, to whose paper the reader is referred for the complete literature of the subject.¹⁹ For the method of estimating methylfurfural the analyst may consult Ellett and Tollens.* The essential principles of the operation are based on the conversion of the pentoses into furfural and of the methylpentoses into methylfurfural by distilling with a strong acid, and the subsequent precipitation and estimation of the fur-

¹⁹ Krug, *Journal of Analytical and Applied Chemistry*, 1893, 7: 68 et seq.

* *Journal für Landwirtschaft*, 1905, 53, [1] 13.

furool and methylfurfurool formed in the first part of the reaction as furfuramid, furfurool hydrazon or furfurool phloroglucid.

The method of conducting the distillation as recommended by the Association of Official Agricultural Chemists is as follows: Place a quantity of the material, chosen so that the weight of phloroglucid obtained shall not exceed 0.300 gram, in a flask, together with 100 cubic centimeters of 12 per cent. hydrochloric acid (1.06 specific gravity) and several pieces of recently heated pumice stone. The arrangement of the apparatus is shown in Fig. 46. The flame of the lamp is so regulated as to secure about

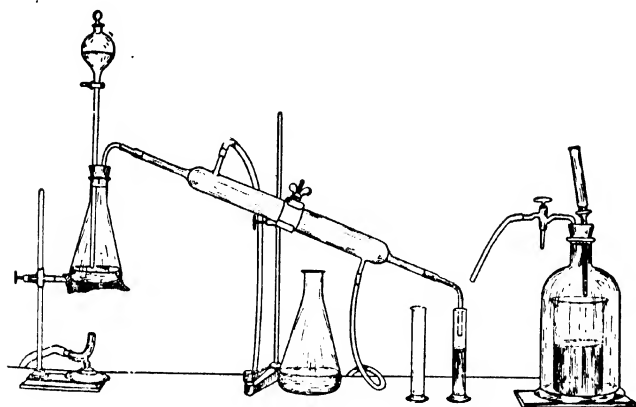


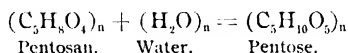
Figure 46. Distilling Apparatus for Pentoses.

two cubic centimeters of distillate per minute or 30 cubic centimeters in a little over 10 minutes.

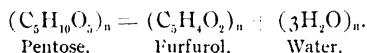
The distillate is received in a graduated cylinder and as soon as thirty cubic centimeters are collected, an equal quantity of hydrochloric acid, of the strength noted, is added to the distilling flask, allowing it to flow in slowly so as not to stop the ebullition. The process is continued until a drop of the distillate gives no sensible reaction for furfurool when tested with anilin acetate. The test is applied as follows: Place a drop of the distillate on a piece of filter paper moistened with anilin acetate.

The presence of furfural will be disclosed by the production of a brilliant red color. Usually about two hours are consumed in the distillation, during which time a little less than 400 cubic centimeters of distillate is obtained.²⁰

The reactions with pentosans probably consist in first splitting up of the molecule into a pentose and the subsequent conversion of the latter into furfural according to the following equations:



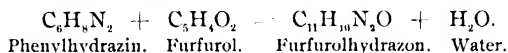
and



195. Determination of Furfural.—The quantity of furfural obtained by the process mentioned above may be determined in several ways.

As Furfuramid.—When ammonia is added to a saturated solution of furfural, furfuramid, $(\text{C}_5\text{H}_4\text{O})_3\text{N}_2$, is formed.²¹ In order to secure the precipitate it is necessary that the furfural be highly concentrated and this can only be accomplished by a tedious fractional distillation. This method, therefore, has little practical value.

As Furfurolylhydrazon.—Furfural is precipitated almost quantitatively, even from dilute solutions, by phenylhydrazin. The reaction is represented by the equation:



²⁰ Flint and Tollens, *Berichte der Deutschen Chemischen Gesellschaft*, 1892, 25: 2912.

²¹ Günther, *Berichte der Deutschen Chemischen Gesellschaft*, 1890, 23: 1751.

As Furfurolphloroglucid.—When a hydrochloric acid solution of phloroglucid is added to a solution containing furfural, precipitation takes place nearly immediately and also quantitatively.

196. Determination of Methylfurfural.—Methylfurfural is precipitated in the same way and by the same reagents as furfural. The methylfurfurolphloroglucid is, however, soluble in alcohol, which the furfurolphloroglucid is not. On this difference the separation can be made.

197. Volumetric Phenylhydrazin Method.—This method for the precipitation of furfural has been used to a great extent, but is now superseded by an improved gravimetric method. Tollens and Gunther²² proposed a volumetric method in which the distillate containing the furfural was neutralized with sodium carbonate and then slightly acidified with acetic acid. A phenylhydrazin solution of known strength was run in until a drop of the liquid showed no reaction for furfural with anilin acetate. Stone²³ proposed a method in which an excess of a known strength solution of phenylhydrazin was added, heated and the excess of this reagent detected by Fehling solution. But these volumetric methods soon gave place to the more exact gravimetric methods. Chalmot and Tollens²⁴ added a solution of phenylhydrazin in acetic acid to the furfural distillate with constant stirring. At the end of half an hour, the furfural hydrazone having separated, as small reddish-brown crystals, the whole mixture was thrown on an asbestos filter and the liquid separated by suction. The hydrazone was dried at 60° and weighed. From this, the quantity of pentosan was determined by a factor.

Factor.—To convert the furfural found into pentoses, the following factors are used:

100 cent. furfural obtained from five grams of pentoses	Multiply for arabinose by	Multiply for xylose by	Multiply for penta-glucoses by
2.5 per cent. or less.....	1.90	1.70	1.67
5.0 per cent. or more.....	2.04	1.90	1.92

²² Berichte der Deutschen Chemischen Gesellschaft, 1890, **23**: 1751.

²³ Journal of Analytical and Applied Chemistry, 1891, **5**: 421.

²⁴ Berichte der Deutschen Chemisches Gesellschaft, **24**: 3575.

In conducting the determination of furfural as given above, Krug observed that the filtrate, after standing for some time, yielded a second precipitate of furfurolylhydrazon. Great difficulty was also experienced in collecting the precipitate upon the filter on account of the persistency with which it stuck to the sides of the vessel in which the precipitation took place.²⁵ In order to avoid these two objections, Krug modified the method as described below.

After the precipitation of the furfurolylhydrazon, it is stirred vigorously, by means of an appropriate mechanical stirrer, for at least half an hour and then allowed to rest for twenty-four hours. On filtering after that length of time the filtrate remains perfectly clear and no further precipitation takes place. After the filtration is complete and the beaker and filtering tube well washed, no attempt is made to detach the part of the filtrate adhering to the beaker but the whole of the precipitate, both that upon the filter and that adhering to the sides of the beaker, is dissolved in strong alcohol, from 30 to 40 cubic centimeters being used. The alcoholic solution is collected in a small weighed flask, the alcohol evaporated at a gentle heat and the last traces of water removed by heating to 60° and blowing a current of dry air through the flask. After weighing the precipitate of furfurolylhydrazon, obtained as above, the calculation of the weight of pentose bodies is accomplished by means of the usual factors. In this form, the method is still used by some chemists, although the method to be described next is more generally practiced.

198. Precipitation by Phloroglucol.—The procedure to be described is a modification of Counciler's²⁶ suggestion, and also Krüger and Tollens.²⁷ It is the method adopted by the Association of Official Agricultural Chemists. The phloroglucol to be used for the precipitation is tested for purity as follows:

Dissolve a small quantity of the phloroglucol in a few drops

²⁵ Journal of Analytical and Applied Chemistry, 1893, 7: 74.

²⁶ Chemiker Zeitung, 1894, 18: 966.

²⁷ Zeitschrift für angewandte Chemie, 1896: heft 2, 33.

of acetic anhydrid, heat almost to boiling, and add a few drops of concentrated sulfuric acid. A violet color indicates the presence of diresorcol. A phloroglucol which gives more than a faint coloration should be purified by the following method:

Heat in a beaker about 300 cubic centimeters of hydrochloric acid (specific gravity, 1.06) and 11 grams of commercial phloroglucol, added in small quantities at a time, stirring constantly until it has almost entirely dissolved. Some impurities may resist solution, but it is unnecessary to dissolve them. Pour the hot solution into a sufficient quantity of the same hydrochloric acid (cold) to make the volume 1,500 cubic centimeters. Allow it to stand at least overnight—better several days—to allow the diresorcol to crystallize out, and filter immediately before using. The solution may turn yellow, but this does not interfere with its usefulness. In using it, add at once the required amount to the distillate.

To the completed distillate of the furfural yielding body, gradually add a quantity of phloroglucol dissolved in 12 per cent. hydrochloric acid and thoroughly stir the resulting mixture. The amount of phloroglucol used should be about double that of the furfural expected. The solution first turns yellow, then green, and very soon an amorphous greenish precipitate appears, which grows rapidly darker, till it finally becomes almost black. Make the solution up to 400 cubic centimeters with 12 per cent. hydrochloric acid, and allow to stand over night.

Filter the amorphous black precipitate into a tared gooch crucible through an asbestos felt, wash carefully with 150 cubic centimeters of water in such a way that the water is not entirely removed from the crucible until the very last, then dry for four hours at the temperature of boiling water, cool and weigh, in a weighing bottle, the increase in weight being reckoned as phloroglucol.

The corresponding weight of pentosan can be obtained from Kibbers tables given below:

DETERMINATION OF PENTOSES AND PENTOSANS FROM
PHLOROGLUCID (KRÖBER).*

1 Phloro- glucid	2 Furfural	3 Arabi- nose	4 Araban	5 Xylose	6 Xylan	7 Pentose	8 Pentosan
0.030	0.0182	0.0391	0.0344	0.0324	0.0285	0.0358	0.0315
0.031	0.0188	0.0402	0.0354	0.0333	0.0293	0.0368	0.0324
0.032	0.0193	0.0413	0.0363	0.0342	0.0301	0.0378	0.0333
0.033	0.0198	0.0424	0.0373	0.0352	0.0309	0.0388	0.0341
0.034	0.0203	0.0435	0.0383	0.0361	0.0317	0.0398	0.0350
0.035	0.0209	0.0446	0.0393	0.0370	0.0326	0.0408	0.0359
0.036	0.0214	0.0457	0.0402	0.0379	0.0334	0.0418	0.0368
0.037	0.0219	0.0468	0.0412	0.0388	0.0342	0.0428	0.0377
0.038	0.0224	0.0479	0.0422	0.0398	0.0350	0.0439	0.0386
0.039	0.0229	0.0490	0.0431	0.0407	0.0358	0.0449	0.0395
0.040	0.0235	0.0501	0.0441	0.0416	0.0366	0.0459	0.0404
0.041	0.0240	0.0512	0.0451	0.0425	0.0374	0.0469	0.0413
0.042	0.0245	0.0523	0.0460	0.0434	0.0385	0.0479	0.0422
0.043	0.0250	0.0534	0.0470	0.0443	0.0390	0.0489	0.0431
0.044	0.0255	0.0545	0.0480	0.0452	0.0398	0.0499	0.0440
0.045	0.0260	0.0556	0.0490	0.0462	0.0406	0.0509	0.0448
0.046	0.0266	0.0567	0.0499	0.0471	0.0414	0.0519	0.0457
0.047	0.0271	0.0578	0.0509	0.0480	0.0422	0.0529	0.0466
0.048	0.0276	0.0589	0.0519	0.0489	0.0430	0.0539	0.0475
0.049	0.0281	0.0600	0.0528	0.0498	0.0438	0.0549	0.0484
0.050	0.0286	0.0611	0.0538	0.0507	0.0446	0.0559	0.0492
0.051	0.0292	0.0622	0.0548	0.0516	0.0454	0.0569	0.0501
0.052	0.0297	0.0633	0.0557	0.0525	0.0462	0.0579	0.0510
0.053	0.0302	0.0644	0.0567	0.0534	0.0470	0.0589	0.0519
0.054	0.0307	0.0655	0.0576	0.0543	0.0478	0.0599	0.0528
0.055	0.0312	0.0666	0.0586	0.0553	0.0486	0.0610	0.0537
0.056	0.0318	0.0677	0.0596	0.0562	0.0494	0.0620	0.0546
0.057	0.0323	0.0688	0.0605	0.0571	0.0502	0.0630	0.0555
0.058	0.0328	0.0699	0.0615	0.0580	0.0510	0.0640	0.0564
0.059	0.0333	0.0710	0.0624	0.0589	0.0518	0.0650	0.0573
0.060	0.0338	0.0721	0.0634	0.0598	0.0526	0.0660	0.0581
0.061	0.0344	0.0732	0.0644	0.0607	0.0534	0.0670	0.0590
0.062	0.0349	0.0743	0.0653	0.0616	0.0542	0.0680	0.0599
0.063	0.0354	0.0754	0.0663	0.0626	0.0550	0.0690	0.0608
0.064	0.0359	0.0765	0.0673	0.0635	0.0558	0.0700	0.0617
0.065	0.0364	0.0776	0.0683	0.0644	0.0567	0.0710	0.0625
0.066	0.0370	0.0787	0.0692	0.0653	0.0575	0.0720	0.0634
0.067	0.0375	0.0798	0.0702	0.0662	0.0583	0.0730	0.0643

* Journal für Landwirtschaft, 1900, 48 : 379.

DETERMINATION OF PENTOSE AND PENTOSANS FROM
PHLOROGLUCID (KRÖBER).—(Continued.)

1 Phloro- glucid	2 Furfural	3 Arabi- nose	4 Araban	5 Xylose	6 Xylan	7 Pentose	8 Pentosan
0.008	0.0380	0.0809	0.0712	0.0672	0.0591	0.0741	0.0652
0.009	0.0385	0.0820	0.0721	0.0681	0.0599	0.0751	0.0661
0.070	0.0390	0.0831	0.0731	0.0690	0.0607	0.0761	0.0670
0.071	0.0396	0.0842	0.0741	0.0699	0.0615	0.0771	0.0679
0.072	0.0401	0.0853	0.0750	0.0708	0.0623	0.0781	0.0688
0.073	0.0406	0.0864	0.0760	0.0717	0.0631	0.0791	0.0697
0.074	0.0411	0.0875	0.0770	0.0726	0.0639	0.0801	0.0706
0.075	0.0416	0.0886	0.0780	0.0736	0.0647	0.0811	0.0714
0.076	0.0422	0.0897	0.0789	0.0745	0.0655	0.0821	0.0722
0.077	0.0427	0.0908	0.0799	0.0754	0.0663	0.0831	0.0731
0.078	0.0432	0.0919	0.0809	0.0763	0.0671	0.0841	0.0740
0.079	0.0437	0.0930	0.0818	0.0772	0.0679	0.0851	0.0749
0.080	0.0442	0.0941	0.0828	0.0781	0.0687	0.0861	0.0758
0.081	0.0448	0.0952	0.0838	0.0790	0.0695	0.0871	0.0767
0.082	0.0453	0.0963	0.0847	0.0799	0.0703	0.0881	0.0776
0.083	0.0458	0.0974	0.0857	0.0808	0.0711	0.0891	0.0785
0.084	0.0463	0.0985	0.0867	0.0817	0.0719	0.0901	0.0794
0.085	0.0468	0.0996	0.0877	0.0827	0.0727	0.0912	0.0803
0.086	0.0474	0.1007	0.0886	0.0836	0.0735	0.0922	0.0812
0.087	0.0479	0.1018	0.0896	0.0845	0.0743	0.0932	0.0821
0.088	0.0484	0.1029	0.0906	0.0854	0.0751	0.0942	0.0830
0.089	0.0489	0.1040	0.0915	0.0863	0.0759	0.0952	0.0838
0.090	0.0494	0.1051	0.0925	0.0872	0.0767	0.0962	0.0847
0.091	0.0499	0.1062	0.0935	0.0881	0.0775	0.0972	0.0856
0.092	0.0505	0.1073	0.0944	0.0890	0.0783	0.0982	0.0865
0.093	0.0510	0.1084	0.0954	0.0900	0.0791	0.0992	0.0874
0.094	0.0515	0.1095	0.0964	0.0909	0.0800	0.1002	0.0883
0.095	0.0520	0.1106	0.0974	0.0918	0.0808	0.1012	0.0891
0.096	0.0525	0.1117	0.0983	0.0927	0.0816	0.1022	0.0899
0.097	0.0531	0.1128	0.0993	0.0936	0.0824	0.1032	0.0908
0.098	0.0536	0.1139	0.1003	0.0946	0.0832	0.1043	0.0917
0.099	0.0541	0.1150	0.1012	0.0955	0.0840	0.1053	0.0926
0.100	0.0546	0.1161	0.1022	0.0964	0.0848	0.1063	0.0935
0.101	0.0551	0.1171	0.1032	0.0973	0.0856	0.1073	0.0944
0.102	0.0557	0.1182	0.1041	0.0982	0.0864	0.1083	0.0953
0.103	0.0562	0.1193	0.1051	0.0991	0.0872	0.1093	0.0962
0.104	0.0567	0.1204	0.1060	0.1000	0.0880	0.1103	0.0971
0.105	0.0572	0.1215	0.1070	0.1010	0.0888	0.1113	0.0976
0.106	0.0577	0.1226	0.1080	0.1019	0.0896	0.1123	0.0988

DETERMINATION OF PENTOSE AND PENTOSANS FROM
PHLOROGLUCID (KRÖBER).—(Continued.)

1 Phloro- glucid	2 Furfural	3 Arabi- nose	4 Araban	5 Xylose	6 Xylan	7 Pentose	8 Pentosan
0.107	0.0582	0.1237	0.1089	0.1028	0.0904	0.1133	0.0997
0.108	0.0588	0.1248	0.1099	0.1037	0.0912	0.1143	0.1006
0.109	0.0593	0.1259	0.1108	0.1046	0.0920	0.1153	0.1015
0.110	0.0598	0.1270	0.1118	0.1055	0.0928	0.1163	0.1023
0.111	0.0603	0.1281	0.1128	0.1064	0.0936	0.1173	0.1032
0.112	0.0608	0.1292	0.1137	0.1073	0.0944	0.1183	0.1041
0.113	0.0614	0.1303	0.1147	0.1082	0.0952	0.1193	0.1050
0.114	0.0619	0.1314	0.1156	0.1091	0.0960	0.1203	0.1059
0.115	0.0624	0.1325	0.1166	0.1101	0.0968	0.1213	0.1067
0.116	0.0629	0.1336	0.1176	0.1110	0.0976	0.1223	0.1076
0.117	0.0634	0.1347	0.1185	0.1119	0.0984	0.1233	0.1085
0.118	0.0640	0.1358	0.1195	0.1128	0.0992	0.1243	0.1094
0.119	0.0645	0.1369	0.1204	0.1137	0.1000	0.1253	0.1103
0.120	0.0650	0.1380	0.1214	0.1146	0.1008	0.1263	0.1111
0.121	0.0655	0.1391	0.1224	0.1155	0.1016	0.1273	0.1120
0.122	0.0660	0.1402	0.1233	0.1164	0.1024	0.1283	0.1129
0.123	0.0665	0.1413	0.1243	0.1173	0.1032	0.1293	0.1138
0.124	0.0671	0.1424	0.1253	0.1182	0.1040	0.1303	0.1147
0.125	0.0676	0.1435	0.1263	0.1192	0.1049	0.1314	0.1156
0.126	0.0681	0.1446	0.1272	0.1201	0.1057	0.1324	0.1165
0.127	0.0686	0.1457	0.1282	0.1210	0.1065	0.1334	0.1174
0.128	0.0691	0.1468	0.1292	0.1219	0.1073	0.1344	0.1183
0.129	0.0697	0.1479	0.1301	0.1228	0.1081	0.1354	0.1192
0.130	0.0702	0.1490	0.1311	0.1237	0.1089	0.1364	0.1201
0.131	0.0707	0.1501	0.1321	0.1246	0.1097	0.1374	0.1210
0.132	0.0712	0.1512	0.1330	0.1255	0.1105	0.1384	0.1219
0.133	0.0717	0.1523	0.1340	0.1264	0.1113	0.1394	0.1227
0.134	0.0723	0.1534	0.1350	0.1273	0.1121	0.1404	0.1236
0.135	0.0728	0.1545	0.1360	0.1283	0.1129	0.1414	0.1244
0.136	0.0733	0.1556	0.1369	0.1292	0.1137	0.1424	0.1253
0.137	0.0738	0.1567	0.1379	0.1301	0.1145	0.1434	0.1262
0.138	0.0743	0.1578	0.1389	0.1310	0.1153	0.1444	0.1271
0.139	0.0748	0.1589	0.1398	0.1319	0.1161	0.1454	0.1280
0.140	0.0754	0.1600	0.1408	0.1328	0.1169	0.1464	0.1288
0.141	0.0759	0.1611	0.1418	0.1337	0.1177	0.1474	0.1297
0.142	0.0764	0.1622	0.1427	0.1346	0.1185	0.1484	0.1306
0.143	0.0769	0.1633	0.1437	0.1355	0.1193	0.1494	0.1315
0.144	0.0774	0.1644	0.1447	0.1364	0.1201	0.1504	0.1324
0.145	0.0780	0.1655	0.1457	0.1374	0.1209	0.1515	0.1333

DETERMINATION OF PENTOSE AND PENTOSANS FROM
PHLOROGLUCID (KRÖBER).—(*Continued.*)

1 Phloro- glucid	2 Furfural	3 Arabi- nose	4 Araban	5 Xylose	6 Xylan	7 Pentose	8 Pentosan
0.146	0.0785	0.1666	0.1466	0.1383	0.1217	0.1525	0.1342
0.147	0.0790	0.1677	0.1476	0.1392	0.1225	0.1535	0.1351
0.148	0.0795	0.1688	0.1486	0.1401	0.1233	0.1545	0.1360
0.149	0.0800	0.1699	0.1495	0.1410	0.1241	0.1555	0.1369
0.150	0.0805	0.1710	0.1505	0.1419	0.1249	0.1565	0.1377
0.151	0.0811	0.1721	0.1515	0.1428	0.1257	0.1575	0.1386
0.152	0.0816	0.1732	0.1524	0.1437	0.1265	0.1585	0.1395
0.153	0.0821	0.1743	0.1534	0.1446	0.1273	0.1595	0.1404
0.154	0.0826	0.1754	0.1544	0.1455	0.1281	0.1605	0.1413
0.155	0.0831	0.1765	0.1554	0.1465	0.1289	0.1615	0.1421
0.156	0.0837	0.1776	0.1563	0.1474	0.1297	0.1625	0.1430
0.157	0.0842	0.1787	0.1573	0.1483	0.1305	0.1635	0.1439
0.158	0.0847	0.1798	0.1583	0.1492	0.1313	0.1645	0.1448
0.159	0.0852	0.1809	0.1592	0.1501	0.1321	0.1655	0.1457
0.160	0.0857	0.1820	0.1602	0.1510	0.1329	0.1665	0.1465
0.161	0.0863	0.1831	0.1612	0.1519	0.1337	0.1675	0.1474
0.162	0.0868	0.1842	0.1621	0.1528	0.1345	0.1685	0.1483
0.163	0.0873	0.1853	0.1631	0.1537	0.1353	0.1695	0.1492
0.164	0.0878	0.1864	0.1640	0.1546	0.1361	0.1705	0.1501
0.165	0.0883	0.1875	0.1650	0.1556	0.1369	0.1716	0.1510
0.166	0.0888	0.1886	0.1660	0.1565	0.1377	0.1726	0.1519
0.167	0.0894	0.1897	0.1669	0.1574	0.1385	0.1736	0.1528
0.168	0.0899	0.1908	0.1679	0.1583	0.1393	0.1746	0.1537
0.169	0.0904	0.1919	0.1688	0.1592	0.1401	0.1756	0.1546
0.170	0.0909	0.1930	0.1698	0.1601	0.1409	0.1766	0.1554
0.171	0.0914	0.1941	0.1708	0.1610	0.1417	0.1776	0.1563
0.172	0.0920	0.1952	0.1717	0.1619	0.1425	0.1786	0.1572
0.173	0.0925	0.1963	0.1727	0.1628	0.1433	0.1796	0.1581
0.174	0.0930	0.1974	0.1736	0.1637	0.1441	0.1806	0.1590
0.175	0.0935	0.1985	0.1746	0.1647	0.1449	0.1816	0.1598
0.176	0.0940	0.1996	0.1756	0.1656	0.1457	0.1826	0.1607
0.177	0.0946	0.2007	0.1765	0.1665	0.1465	0.1836	0.1616
0.178	0.0951	0.2018	0.1775	0.1674	0.1473	0.1846	0.1625
0.179	0.0956	0.2029	0.1784	0.1683	0.1481	0.1856	0.1634
0.180	0.0961	0.2039	0.1794	0.1692	0.1489	0.1866	0.1642
0.181	0.0966	0.2050	0.1804	0.1701	0.1497	0.1876	0.1651
0.182	0.0971	0.2061	0.1813	0.1710	0.1505	0.1886	0.1660
0.183	0.0977	0.2072	0.1823	0.1719	0.1513	0.1896	0.1669
0.184	0.0982	0.2082	0.1832	0.1728	0.1521	0.1906	0.1678

DETERMINATION OF PENTOSES AND PENTOSANS FROM
PHLOROGLUCID (KRÖBER).—(Continued.)

1 Phloro- glucid	2 Furfural	3 Arabi- nose	4 Araban	5 Xylose	6 Xylan	7 Pentose	8 Pentosan
0.185	0.0987	0.2093	0.1842	0.1738	0.1529	0.1916	0.1686
0.186	0.0992	0.2104	0.1851	0.1747	0.1537	0.1926	0.1695
0.187	0.0997	0.2115	0.1861	0.1756	0.1545	0.1936	0.1704
0.188	0.1003	0.2126	0.1870	0.1765	0.1553	0.1946	0.1712
0.189	0.1008	0.2136	0.1880	0.1774	0.1561	0.1955	0.1721
0.190	0.1013	0.2147	0.1889	0.1783	0.1569	0.1965	0.1729
0.191	0.1018	0.2158	0.1899	0.1792	0.1577	0.1975	0.1738
0.192	0.1023	0.2168	0.1908	0.1801	0.1585	0.1985	0.1747
0.193	0.1028	0.2179	0.1918	0.1810	0.1593	0.1995	0.1756
0.194	0.1034	0.2190	0.1927	0.1819	0.1601	0.2005	0.1764
0.195	0.1039	0.2201	0.1937	0.1829	0.1609	0.2015	0.1773
0.196	0.1044	0.2212	0.1946	0.1838	0.1617	0.2025	0.1782
0.197	0.1049	0.2222	0.1956	0.1847	0.1625	0.2035	0.1791
0.198	0.1054	0.2233	0.1965	0.1856	0.1633	0.2045	0.1800
0.199	0.1059	0.2244	0.1975	0.1865	0.1641	0.2055	0.1808
0.200	0.1065	0.2255	0.1984	0.1874	0.1649	0.2065	0.1817
0.201	0.1070	0.2266	0.1994	0.1883	0.1657	0.2075	0.1826
0.202	0.1075	0.2276	0.2003	0.1892	0.1665	0.2085	0.1835
0.203	0.1080	0.2287	0.2013	0.1901	0.1673	0.2095	0.1844
0.204	0.1085	0.2298	0.2022	0.1910	0.1681	0.2105	0.1853
0.205	0.1090	0.2309	0.2032	0.1920	0.1689	0.2115	0.1861
0.206	0.1096	0.2320	0.2041	0.1929	0.1697	0.2125	0.1869
0.207	0.1101	0.2330	0.2051	0.1938	0.1705	0.2134	0.1878
0.208	0.1106	0.2341	0.2060	0.1947	0.1713	0.2144	0.1887
0.209	0.1111	0.2352	0.2069	0.1956	0.1721	0.2154	0.1896
0.210	0.1116	0.2363	0.2079	0.1965	0.1729	0.2164	0.1904
0.211	0.1121	0.2374	0.2089	0.1975	0.1737	0.2174	0.1913
0.212	0.1127	0.2384	0.2098	0.1984	0.1745	0.2184	0.1922
0.213	0.1132	0.2395	0.2108	0.1993	0.1753	0.2194	0.1931
0.214	0.1137	0.2406	0.2117	0.2002	0.1761	0.2204	0.1940
0.215	0.1142	0.2417	0.2127	0.2011	0.1770	0.2214	0.1948
0.216	0.1147	0.2428	0.2136	0.2020	0.1778	0.2224	0.1957
0.217	0.1152	0.2438	0.2146	0.2029	0.1786	0.2234	0.1966
0.218	0.1158	0.2449	0.2155	0.2038	0.1794	0.2244	0.1974
0.219	0.1163	0.2460	0.2165	0.2047	0.1802	0.2254	0.1983
0.220	0.1168	0.2471	0.2174	0.2057	0.1810	0.2264	0.1992
0.221	0.1173	0.2482	0.2184	0.2066	0.1818	0.2274	0.2001
0.222	0.1178	0.2492	0.2193	0.2075	0.1826	0.2284	0.2010
0.223	0.1183	0.2503	0.2203	0.2084	0.1834	0.2294	0.2019
0.224	0.1189	0.2514	0.2212	0.2093	0.1842	0.2304	0.2028

DETERMINATION OF PENTOSE AND PENTOSANS FROM
PHLOROGLUCID (KRÖBER).—*Continued*.

1 Phloro- glucid	2	3 Arabi- nose	4	5	6	7	8
Furfural	Araban	Xylose	Xylan	Pentose	Pentosan		
0.225	0.1194	0.2525	0.2222	0.2102	0.1850	0.2314	0.2037
0.226	0.1199	0.2536	0.2232	0.2111	0.1858	0.2324	0.2046
0.227	0.1204	0.2546	0.2241	0.2121 ¹	0.1866	0.2334	0.2054
0.228	0.1209	0.2557	0.2251	0.2130	0.1874	0.2344	0.2063
0.229	0.1214	0.2568	0.2260	0.2139	0.1882	0.2354	0.2072
0.230	0.1220	0.2579	0.2270	0.2148	0.1890	0.2364	0.2081
0.231	0.1225	0.2590	0.2280	0.2157	0.1898	0.2374	0.2089
0.232	0.1230	0.2600	0.2289	0.2166	0.1906	0.2383	0.2097
0.233	0.1235	0.2611	0.2299	0.2175	0.1914	0.2393	0.2106
0.234	0.1240	0.2622	0.2308	0.2184	0.1922	0.2403	0.2115
0.235	0.1245	0.2633	0.2318	0.2193	0.1930	0.2413	0.2124
0.236	0.1251	0.2644	0.2327	0.2202	0.1938	0.2423	0.2132
0.237	0.1256	0.2654	0.2337	0.2211	0.1946	0.2433	0.2141
0.238	0.1261	0.2665	0.2346	0.2220	0.1954	0.2443	0.2150
0.239	0.1266	0.2676	0.2356	0.2229	0.1962	0.2453	0.2159
0.240	0.1271	0.2687	0.2365	0.2239	0.1970	0.2463	0.2168
0.241	0.1276	0.2698	0.2375	0.2248	0.1978	0.2473	0.2176
0.242	0.1281	0.2708	0.2384	0.2257	0.1986	0.2483	0.2185
0.243	0.1287	0.2719	0.2394	0.2266	0.1994	0.2493	0.2194
0.244	0.1292	0.2730	0.2403	0.2275	0.2002	0.2503	0.2203
0.245	0.1297	0.2741	0.2413	0.2284	0.2010	0.2513	0.2212
0.246	0.1302	0.2752	0.2422	0.2293	0.2018	0.2523	0.2220
0.247	0.1307	0.2762	0.2432	0.2302	0.2026	0.2533	0.2229
0.248	0.1312	0.2773	0.2441	0.2311	0.2034	0.2543	0.2238
0.249	0.1318	0.2784	0.2451	0.2320	0.2042	0.2553	0.2247
0.250	0.1323	0.2795	0.2460	0.2330	0.2050	0.2563	0.2256
0.251	0.1328	0.2806	0.2470	0.2339	0.2058	0.2573	0.2264
0.252	0.1333	0.2816	0.2479	0.2348	0.2066	0.2582	0.2272
0.253	0.1338	0.2827	0.2489	0.2357	0.2074	0.2592	0.2281
0.254	0.1343	0.2838	0.2498	0.2366	0.2082	0.2602	0.2290
0.255	0.1349	0.2849	0.2508	0.2375	0.2090	0.2612	0.2299
0.256	0.1354	0.2860	0.2517	0.2384	0.2098	0.2622	0.2307
0.257	0.1359	0.2870	0.2526	0.2393	0.2106	0.2632	0.2316
0.258	0.1364	0.2881	0.2536	0.2402	0.2114	0.2642	0.2325
0.259	0.1369	0.2892	0.2545	0.2411	0.2122	0.2652	0.2334
0.260	0.1374	0.2903	0.2555	0.2420	0.2130	0.2662	0.2343
0.261	0.1380	0.2914	0.2565	0.2429	0.2138	0.2672	0.2351
0.262	0.1385	0.2924	0.2574	0.2438	0.2146	0.2681	0.2359
0.263	0.1390	0.2935	0.2584	0.2447	0.2154	0.2691	0.2368
0.264	0.1395	0.2946	0.2593	0.2456	0.2162	0.2701	0.2377

DETERMINATION OF PENTOSE AND PENTOSANS FROM
PHLOROGLUCID (KRÖBER).—(Continued.)

1 Phloro- glucid	2 Furfural	3 Arabi- nose	4 Araban	5 Xylose	6 Xylan	7 Pentose	8 Pentosan
0.265	0.1400	0.2957	0.2603	0.2465	0.2170	0.2711	0.2385
0.266	0.1405	0.2968	0.2612	0.2474	0.2178	0.2721	0.2394
0.267	0.1411	0.2978	0.2622	0.2483	0.2186	0.2731	0.2403
0.268	0.1416	0.2989	0.2631	0.2492	0.2194	0.2741	0.2412
0.269	0.1421	0.3000	0.2641	0.2502	0.2202	0.2751	0.2421
0.270	0.1426	0.3011	0.2650	0.2511	0.2210	0.2761	0.2429
0.271	0.1431	0.3022	0.2660	0.2520	0.2218	0.2771	0.2438
0.272	0.1436	0.3032	0.2669	0.2529	0.2226	0.2781	0.2447
0.273	0.1442	0.3043	0.2679	0.2538	0.2234	0.2791	0.2456
0.274	0.1447	0.3054	0.2688	0.2547	0.2242	0.2801	0.2465
0.275	0.1452	0.3065	0.2698	0.2556	0.2250	0.2811	0.2473
0.276	0.1457	0.3076	0.2707	0.2565	0.2258	0.2821	0.2482
0.277	0.1462	0.3086	0.2717	0.2574	0.2266	0.2830	0.2490
0.278	0.1467	0.3097	0.2726	0.2583	0.2274	0.2840	0.2499
0.279	0.1473	0.3108	0.2736	0.2592	0.2282	0.2850	0.2508
0.280	0.1478	0.3119	0.2745	0.2602	0.2290	0.2861	0.2517
0.281	0.1483	0.3130	0.2755	0.2611	0.2298	0.2871	0.2526
0.282	0.1488	0.3140	0.2764	0.2620	0.2306	0.2880	0.2534
0.283	0.1493	0.3151	0.2774	0.2629	0.2314	0.2890	0.2543
0.284	0.1498	0.3162	0.2783	0.2638	0.2322	0.2900	0.2552
0.285	0.1504	0.3173	0.2793	0.2647	0.2330	0.2910	0.2561
0.286	0.1509	0.3184	0.2802	0.2656	0.2338	0.2920	0.2570
0.287	0.1514	0.3194	0.2812	0.2665	0.2346	0.2930	0.2578
0.288	0.1519	0.3205	0.2821	0.2674	0.2354	0.2940	0.2587
0.289	0.1524	0.3216	0.2831	0.2683	0.2362	0.2950	0.2596
0.290	0.1529	0.3227	0.2840	0.2693	0.2370	0.2960	0.2605
0.291	0.1535	0.3238	0.2850	0.2702	0.2378	0.2970	0.2614
0.292	0.1540	0.3248	0.2859	0.2711	0.2386	0.2980	0.2622
0.293	0.1545	0.3259	0.2868	0.2720	0.2394	0.2990	0.2631
0.294	0.1550	0.3270	0.2878	0.2729	0.2402	0.3000	0.2640
0.295	0.1555	0.3281	0.2887	0.2738	0.2410	0.3010	0.2649
0.296	0.1560	0.3292	0.2897	0.2747	0.2418	0.3020	0.2658
0.297	0.1566	0.3302	0.2906	0.2756	0.2426	0.3030	0.2666
0.298	0.1571	0.3313	0.2916	0.2765	0.2434	0.3040	0.2675
0.299	0.1576	0.3324	0.2925	0.2774	0.2442	0.3050	0.2684
0.300	0.1581	0.3335	0.2935	0.2784	0.2450	0.3060	0.2693

Browne's factors, calculated from Kröber's tables in which
a = weight of phloroglucid obtained are as follows:

²⁸ Bulletin 107, Revised 1908, Bureau of Chemistry, 55.

For weight of phloroglucid from 0.03 to 0.300 gram.

$$\text{Furfurol} = (a + 0.0052) \times 0.5185.$$

$$\text{Pentoses} = (a + 0.0052) \times 1.0075.$$

$$\text{Pentosans} = (a + 0.0052) \times 0.8866.$$

199. Method for Methylpentosans in Presence of Pentosans.—To Ellett and Tollens²⁹ is due the credit of working out the following method. It has been modified somewhat and in its present form is the one adopted by the Association of Official Agricultural Chemists.

Proceed as in the determination of pentosans given above until the phloroglucid precipitate has been dried for four hours and weighed. Place the gooch crucible containing this precipitate in a 100 cubic centimeter beaker and pour into the gooch 30 cubic centimeters of 95 per cent. alcohol heated to 60 degrees. Place the beaker for 10 minutes in a water bath heated to 60 degrees. Remove the beaker and gooch and draw off from the gooch with a suction pump all alcohol remaining therein. Repeat this alternate extraction and sucking dry of the precipitate from three to five times, according to the color of the filtrate obtained. After the final extraction place the gooch crucible in a water oven and dry four hours, making the final weighing in a closely stoppered glass weighing bottle, as described in the method for pentosans. This weight is weight of crucible plus furfurolyphloroglucid, the methylfurfurol phloroglucid having dissolved.

The difference in weight between the furfurolyphloroglucid plus methylfurfurolyphloroglucid first obtained and the furfurolyphloroglucid remaining after extraction with alcohol, represents the amount of methylfurfurolyphloroglucid present, but as furfurolyphloroglucid is slightly soluble, or some other substance is present in the precipitate that is soluble, a correction of 0.0037 gram has to be made. This amount is subtracted from the amount of methylfurfurolyphloroglucid obtained. This corrected figure is then used for the calculation of the original pentose (as rhamnose). The table given below can be used for obtaining the weight of the various methyl-pentosans from the

²⁹ Bureau of Chemistry, Bulletin 105, 1907: 115-116.

TABLE FOR METHYLFURFUROL, PHLOROGLUCID TO METHYLPENTOSANS.³⁰

1	2	3	4	5	6
Methyl- Phloroglucid g.	Fukose g.	Fukosan (col. 2 ÷ 0.89)	Rhamnose according Ellett & Tollens	Rhamnosan (col. 4 ÷ 0.8)	Methyl- pentosan (average of col. 3 & 5)
0.010	0.0260	0.0231	0.0266	0.0213	0.0222
0.011	0.0284	0.0253	0.0279	0.0223	0.0238
0.012	0.0307	0.0274	0.0295	0.0236	0.0255
0.013	0.0331	0.0295	0.0311	0.0249	0.0272
0.014	0.0354	0.0315	0.0327	0.0262	0.0288
0.015	0.0377	0.0336	0.0343	0.0274	0.0305
0.016	0.0400	0.0356	0.0359	0.0287	0.0321
0.017	0.0423	0.0376	0.0375	0.0300	0.0338
0.018	0.0445	0.0396	0.0391	0.0313	0.0354
0.019	0.0467	0.0416	0.0407	0.0326	0.0371
0.020	0.0489	0.0435	0.0423	0.0338	0.0386
0.021	0.0510	0.0454	0.0438	0.0350	0.0402
0.022	0.0532	0.0473	0.0454	0.0363	0.0418
0.023	0.0553	0.0492	0.0469	0.0375	0.0433
0.024	0.0574	0.0511	0.0485	0.0388	0.0449
0.025	0.0594	0.0529	0.0500	0.0400	0.0462
0.026	0.0614	0.0547	0.0516	0.0413	0.0480
0.027	0.0634	0.0565	0.0531	0.0425	0.0495
0.028	0.0654	0.0583	0.0547	0.0438	0.0510
0.029	0.0674	0.0600	0.0562	0.0450	0.0525
0.030	0.0693	0.0617	0.0578	0.0462	0.0539
0.031	0.0712	0.0634	0.0593	0.0474	0.0554
0.032	0.0731	0.0651	0.0609	0.0487	0.0569
0.033	0.0750	0.0668	0.0624	0.0499	0.0584
0.034	0.0768	0.0684	0.0639	0.0511	0.0598
0.035	0.0786	0.0700	0.0655	0.0524	0.0612
0.036	0.0804	0.0716	0.0670	0.0536	0.0626
0.037	0.0822	0.0732	0.0685	0.0548	0.0640
0.038	0.0839	0.0747	0.0700	0.0560	0.0654
0.039	0.0857	0.0764	0.0716	0.0573	0.0668
0.040	0.0874	0.0778	0.0731	0.0585	0.0681
0.041	0.0890	0.0792	0.0747	0.0598	0.0695
0.042	0.0907	0.0807	0.0761	0.0609	0.0708
0.043	0.0923	0.0821	0.0775	0.0620	0.0721
0.044	0.0939	0.0836	0.0790	0.0632	0.0734
0.045	0.0954	0.0850	0.0803	0.0644	0.0747
0.046	0.0970	0.0863	0.0820	0.0656	0.0759
0.047	0.0985	0.0877	0.0835	0.0668	0.0772
0.048	0.1000	0.0890	0.0849	0.0679	0.0785
0.049	0.1015	0.0903	0.0864	0.0691	0.0797
0.050	0.1029	0.0916	0.0879	0.0703	0.0809

³⁰ Journal für Landwirtschaft, 1907, 55, P. 269.

weight of methylfurfurophloroglucid obtained or the following formula for rhamnose can be used:

$$\text{Rhamnose} = (\text{Ph}) (1.65) - (\text{Ph})^2 (1.84) + 0.010.$$

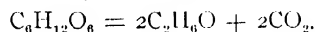
in which "Ph" equals the weight of methylfurfurophloroglucid; rhamnosan equals rhamnose multiplied by 0.8.

Example: The methylphloroglucid weighs 0.0766 gram.

$$\begin{aligned} \text{Then } 0.0766 \times 1.65 - (0.0766^2) \times 1.84 + 0.010 = \\ 0.12639 - 0.010796 + 0.10 = 0.1255 \text{ gram rhamnose.} \end{aligned}$$

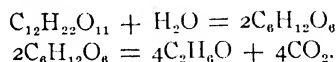
To obtain the weight of pentosans, subtract the final corrected weight of methylfurfurophloroglucid from the weight of the mixture of methylfurfurophloroglucid and furfurophloroglucid and calculate according to Kröber's table, or according to the formulas given for pentosans.

200. Estimation of Sugars by Fermentation.—When a solution of a hexose sugar is subjected to the action of certain ferments a decomposition of the molecule takes place with the production of carbon dioxid and various alcohols and organic acids. Under the action of the ferment of yeast *Saccharomyces cerevisiae* the sugar yields theoretically only carbon dioxid and ethyl alcohol, as represented by the equation:



The theoretical quantities of alcohol and carbon dioxid obtained according to this equation are 51.11 per cent. of alcohol and 48.89 per cent. of carbon dioxid.

When the yeast ferment acts on cane sugar the later first suffers inversion, and the molecules of dextrose and levulose produced are subsequently converted into alcohol and carbon dioxid as represented below:



Cane sugar, plus the water of hydrolysis, will yield theoretically 53.8 per cent. of alcohol and 51.5 per cent. of carbon dioxid.

In practice the theoretical proportions of alcohol and carbon dioxid are not obtained because of the difficulty of excluding other fermentative action, resulting in the formation especially

of succinic acid and glycerol. Moreover, a part of the sugar is consumed by the yeast cells to secure their proper growth and development. In all only about 95 per cent. of the sugar can be safely assumed as entering into the production of alcohol. About 48.5 per cent. of alcohol is all that may be expected of the weight of dextrose or invert sugar used. Only sugars containing three molecules of carbon or some multiple thereof are fermentable. Thus the trioses, hexoses, nonoses, etc., are susceptible of fermentation, while the tetroses, pentoses, etc., are not.

201. Estimating Alcohol.—In the determination of sugar by fermentation, a rather dilute solution not exceeding 10 per cent. should be used. A quantity of pure yeast, equivalent to four or five per cent. of the sugar used, is added, and the contents of the vessel, after being well shaken, exposed to a temperature of from 25° to 30° until the fermentation has ceased, which will be usually in from twenty-four to thirty-six hours. The alcohol is then determined in the residue by the methods given hereafter.

The weight of the alcohol obtained multiplied by 100 and divided by 48.5, will give the weight of the hexose reducing sugar which has been fermented. Ninety-five parts of sucrose will give 100 parts of invert sugar.

Example.—Let the weight of alcohol obtained be 0.625 gram. Then $0.625 \times 100 \div 48.5 = 1.289$ grams, the weight of the hexose, which has been fermented; 1.289 grams of dextrose or levulose correspond to 1.225 of sucrose.

202. Estimating Carbon Dioxid.—The sugar may also be determined by estimating the amount of carbon dioxid produced during the fermentation. For this purpose the mixture of sugar solution and yeast, prepared as above mentioned, is placed in a flask whose stopper carries two tubes, one of which introduces air free of carbon dioxid into the contents of the flask, and the other conducts the evolved carbon dioxid into the absorption bulbs. In passing to the absorption bulbs the carbon dioxid is freed of moisture by passing through another set of bulbs filled with strong sulfuric acid. During the fermentation, the carbon

dioxid is forced through the bulbs by the pressure produced, or better, a slow current of air is aspirated through the whole apparatus. The aspiration is continued after the fermentation has ceased, until all the carbon dioxid is expelled. Towards the end, the contents of the flask may be heated to near the boiling-point. The increase of the weight of the potash bulbs will give the weight of carbon dioxid obtained. A hexose reducing sugar will yield about 46.5 per cent. of its weight of carbon dioxid. The calculation is made as suggested for the alcohol process.

203. Separation of Soluble Carbohydrates by Yeasts.—The fermentation process has been farther applied by chemists to the study of sugars in mixtures. It has been found that certain yeasts will act upon certain sugars and not upon others. Then by applying these yeasts to sugars, solutions one after the other of the sugars can be fermented out and determination of the quantity of the individual sugar be made. The following table shows the selection activity of some of the yeasts. This is taken from Lindner³¹ and the different yeasts are obtainable from the Institute für Gärungsgewerbe in Berlin.

TABLE (CHEMIE DER MENSCHLICHEN NAHRUNGS-UND GENUSSMITTEL, 1910, 3:425), BY DR. J. KÖNIG.

Name of the yeast	Glu- cose	Fruc- tose	Mal- tose	Suc- rose	Dex- trin
<i>Saccharomyces apiculatus</i> ...	+	+	—	—	—
<i>Torula pulcherrima</i>	+	+	—	—	—
<i>Torula aus Mazun</i>	+	+	—	—	—
<i>Sacch. Marxianus</i>	+	+	—	+	—
<i>Sacch. Ludwigü</i>	+	+	—	+	—
<i>Hefe aus Kissleytschi</i>	+	+	—	+	—
<i>Hefe aus Armenischem Mazum</i>	+	+	—	+	—
<i>Hefe aus Zuckerrohrmelasse</i> .	+	+	+	+	—
<i>Sacch. Saaz, untergärig</i>	+	+	+	+	—
<i>Hefe aus Danziger Jopenbier</i> .	+	+	+	+	—
<i>Schizo-Sacch. Pombe</i>	+	+	+	+	+
<i>Sacch. Logos</i>	+	+	+	+	+
<i>Sachsia Suaveolens</i>	+	+	+	+	+
<i>Monilia variabiles</i>	+	+	+	+	+

+ Means attacked by the yeast, while — not fermented by the yeast.

In practice 58 grams of the dry sugar containing material is

³¹ Wochenschrift für Brauerei, 1900, 17:49-51.

dissolved in about 100 cubic centimeters of Raulin's culture solution and the whole placed in a fermentation flask. This is allowed to stand depending on the activity of the yeast, from one to six days at a temperature of 30 to 32°. With fruit juices sometimes a longer time is necessary. Weighing the fermentation flask from time to time will give a rough estimation of the completeness of the operation and also the percentage of the sugar being fermented. After fermentation the residue can be tested for the other sugars.

The formula for Raulin's culture solution is:

Ammonium tartrate.....	4.0 grams.
Ammonium nitrate.....	4.0 grams.
Ammonium phosphate.....	0.6 grams.
Ammonium sulfate.....	0.25 grams.
Potassium carbonate.....	0.6 grams.
Potassium silicate.....	0.4 grams.
Magnesium sulfate.....	0.4 grams.
Ferric sulfate.....	0.07 grams.
Zinc sulfate.....	0.07 grams.
Water.....	1500 cc.

After solution, it should be sterilized.

König and Hörmann³² have published a full report on the separation of glucose, fructose, sucrose, and maltose also dextrin from dextrose, fructose, sucrose and dextrin from glucose and fructose, by the use of these yeasts. A discussion of this subject would naturally come up later under sugar separations, but it is doubtful whether more than a passing glance can be taken of this subject in the scope of our work. The principle of the method is good and it seems probable that much advancement along this line will take place in the years to come.

204. Enzymes.—While discussing this phase of the subject, a word should be introduced here regarding a class of substances found in the cells of plant and animal life, and capable of extraction, which are able to produce certain definite chemical reactions and which go under the name of enzymes. The chemical composition of these is not known and so far they are not capable

³² Zeitschrift für Untersuchung der Nahrungs-und Genussmittel, 1907, 13: 113.

of isolation in a perfectly free state. Their activity is of a catalytic character and for this reason a very small amount of enzyme can produce a comparatively great proportion of chemical change. There are, however, enzymes whose activity is somewhat different, notably the oxydases; but in general it may be said that enzymic action is hydrolytic; that is, it consists in splitting off simpler substances with a simultaneous absorption of water.

Enzymes differ greatly as regards the chemical reactions which they are capable of causing, and because of this fact they may be divided into several classes. Sugar splitting enzymes, fat splitting enzymes, protein splitting enzymes, oxidation and alcohol forming enzymes. A name for each class in order, would be carbohydrate enzymes, lipases, proteolytic enzymes, oxydases and zymases.

The enzymes under the carbohydrate splitting class generally bear the name of the sugar upon which they especially act with the "ose" changed to "ase," as for instance, lactase and maltase. However, we have invertase which splits sucrose and diastase which splits starch.

205. Precipitation of Sugars by Combination with the Earthy Bases.—Most sugars combine in varying proportions with metallic oxids especially those of calcium, strontium and barium. Sucrose furnishes definite crystalline compounds with these bases in such a way as to form the groundwork of several technical processes in the separation of that substance in molasses from its normally and abnormally associated compounds. These processes have little use as analytical methods, but are of great value, as mentioned, from a technical point of view.

Fructose has been prepared commercially from invert sugar by forming the calcium fructosate and then decomposing it. The lead salts of some sugars, especially in ammonia solutions, serve to separate these sugars from various compounds as raffinose from molasses.

206. Barium Saccharate.—This compound is formed by mixing the aqueous solutions of barium hydroxid and sugar. The sac-

charate separates in bright crystalline plates or needles from the warm solution, as $C_{12}H_{22}O_{11}BaO$. One part of this precipitate is soluble in about 45 parts of water, both at 15° and 100° .

207. Strontium Saccharates.—Both the mono- and distrontium saccharates are known. *viz.*, $C_{12}H_{22}O_{11}SrO + 5H_2O$ and $C_{12}H_{22}O_{11}2SrO$.

The monosalt is secured by adding a saturated strontium hydroxid solution to a saturated sucrose solution and allowing to stand in the cold at least 24 hours

The disaccharate is precipitated as a granular substance when strontium hydroxid solution is added to a boiling sugar solution in the proportion of two to three molecules of strontium to that of sugar. The reaction is extensively used in Germany in separating the sugar from beet molasses.

In the laboratory but little success has been had in using barium hydroxid as a precipitating agent, and therefore the reactions mentioned above are of little value for analytical purposes. In separating sugar from vegetable fibers and seeds, however, the treatment with strontium hydroxid is especially valuable, the sugar being subsequently recovered in a free state by breaking up the saccharate with carbon dioxid.

208. Calcium Saccharates.—Three calcium saccharates are known in which one molecule of sugar is combined with one, two and three molecules of lime respectively.

The monosaccharate is obtained by mixing the sugar and lime in the proper proportion and precipitating by adding alcohol. The formula for this generally given is $C_{12}H_{22}O_{11}.CaO + 2H_2O$.

The precipitate is partly granular and partly jelly-like, and is soluble in cold water. The dicalcium compound ($C_{12}H_{22}O_{11}.2CaO$) is obtained in the same way and has similar properties. Both, on boiling with water form the trisaccharate and free sugar.

The tricalcium saccharate is the most important of these compounds, and may be obtained directly by mixing freshly burned and finely ground lime (CaO) with a very cold dilute solution of sugar.

The compound crystallizes with three or four molecules of water. When precipitated as described above, however, it has a granular, nearly amorphous structure, and the process is used in the separation of sugar from beet molasses in this country under the name of the Steffens molasses process.

In general it may be said that the compounds of sucrose and other sugars with the earthly bases have technical rather than chemical importance. The compounds which these bodies form with phenylhydrazin on the other hand are of high significance chemically.

209. Cobaltous Nitrate Test for Sucrose.—Sucrose in solution may be distinguished from other sugars by the amethyst violet color which it imparts to a solution of cobaltous nitrate. This reaction was first described by Reich, in 1856, but has only lately been worked out in detail. The test is applied as follows:

To about 15 cubic centimeters of the sugar solution add five cubic centimeters of a five per cent. solution of cobaltous nitrate. After thoroughly mixing the two solutions, add two cubic centimeters of a 50 per cent. solution of sodium hydroxid. Pure sucrose gives by this treatment an amethyst violet color, which is permanent. Pure dextrose gives a turquoise blue color which soon passes into a light green. When the two sugars are mixed the coloration by the sucrose is the predominant one, and one part of sucrose in nine parts of dextrose can be distinguished. If the sucrose be mixed with impurities such as gum arabic or dextrin, they should be precipitated by alcohol or basic lead acetate, before the application of the test. Dextrin may be thrown out by treatment of the solution with barium hydroxid and ammoniacal lead acetate. It is said that the reaction may also be applied to the detection of cane sugar in wines, after they are thoroughly decolorized by means of lead acetate and bone-black. Likewise the presence of added sucrose to milk, either in the fresh or condensed state, may also be detected after the disturbing matters are thrown out with lead acetate. The amethyst violet coloration

with sucrose is practically permanent. On boiling the color is made slightly bluish, but is restored to the original tint on cooling. Dextrose gives at first a fine blue color which in the course of two hours passes into a pale green. A slight flocculent precipitate is noticed in the tube containing the dextrose. Maltose and lactose act very much as dextrose, but in the end do not give so fine a green color. If the solutions containing dextrose, lactose and maltose be boiled, the original color is destroyed and a yellow-green color takes its place. The reaction is one which promises to be of considerable practical value to analysts, as it may be applied for the qualitative detection of sucrose in seeds and other vegetable products.³³

210. The Dextrose Group.—In case the carbohydrate in question shows a right-handed rotation and the absence of sucrose is established by the polariscopic observation described above, the presence of the dextrose group may be determined by the following test.*

Five grams of the carbohydrate are oxidized by boiling with from 20 to 30 cubic centimeters of nitric acid of 1.15 specific gravity, and then at gentle heat evaporated to dryness with stirring. If much mucic acid be present, as will be the case if the original matter contained lactose some water is added and the mixture well stirred, and again evaporated to dryness to expel all nitric acid. The residue should be of a brown color. The mass is again mixed with a little water and the acid reaction neutralized by treating with fine-ground potassium carbonate. The carbonate should be added in slight excess and acetic acid added to the alkaline mixture, which is concentrated by evaporation and allowed to stand a few days. At the end of this time potassium saccharate has formed and is separated from the mother liquid by pouring on a porous porcelain plate. The residue is collected, dissolved in a little water and again allowed to crystallize, when it is collected on a porous plate, as before, and washed by means of an atomizer with a little aqueous spray

³³ Papasogli, *Bulletin de l'Association der Chimistes de Sucrerie et de Distillerie*, 1895, **13**: 68.

* Gans und Tollens, *Zeitschrift des Vereins für die Rübenzucker-Industrie*, 1888, **38**: neuer folge **25**: 1126.

until it is pure white and free of any oxalic acid. The residual acid potassium saccharate may be weighed after drying and then converted into the silver salt. The potash salt for this purpose is dissolved in water, neutralized with ammonia and precipitated with a solution of silver nitrate. The precipitate is well stirred, collected on a gooch and washed and dried in a dark place. It contains 50.94 per cent. of silver. All sugars which contain the dextrose group yield silver saccharate when treated as above described. Inulin, sorbose, arabinose and galactose yield no saccharic acid under this treatment, and thus it is shown that they contain no dextrose group. Milk sugar, maltose, the dextrans, raffinose and sucrose yield saccharic acid when treated as above and therefore all contain the dextrose group.

211. Levulose.—The levulose group of sugars, wherever it occurs, when oxidized with nitric acid, gives rise to tartaric, racemic, glycolic and oxalic acids, which are not characteristic, being produced also by the oxidation of other carbohydrates. A more distinguishing test is afforded by the color reactions produced with resorcin.

Resorcin Test:—The reagent is prepared by dissolving half a gram of resorcin in 30 cubic centimeters each of water and strong hydrochloric acid. To the sugar solution under examination an equal volume of strong hydrochloric acid is added, and then a few drops of the reagent. The mixture is gently warmed, and in the presence of levulose develops a fire-red color.⁸⁴ Dextrose, lactose, mannose and the pentoses do not give the coloration, but it is produced by sorbose in a striking degree, and also by sucrose and raffinose since these sugars contain the levulose group.

212. Galactose.—The galactose which arises from the hydrolysis of milk sugar is readily recognized by the mucic acid which it gives an oxidation with nitric acid.⁸⁵ The analytical work

⁸⁴ *Berichte der Deutschen Chemischen Gesellschaft*, 1887, **20**: 303; *Zeitschrift des Vereins für die Rübenzucker-Industrie*, 1891, **41**, neuer folge 28: 895.

⁸⁵ *Zeitschrift des Vereins für die Rübenzucker-Industrie*, 1891, **41**, neuer folge 25: 891.

is conducted as follows: The body containing galactose or galactan is placed in a beaker with about 60 cubic centimeters of nitric acid of 1.15 specific gravity for each five grams of the sample used. The beaker is placed on a steam-bath and heated, with frequent stirring, until two-thirds of the nitric acid have been evaporated. The residual mixture is allowed to stand over night and the following morning is treated with 10 cubic centimeters of water, allowed to stand for 24 hours, filtered through a gouch, and the collected matter washed with 25 cubic centimeters of water, dried at 100° and weighed. The mucic acid collected in this way will amount to about 37 per cent. of the milk sugar or 75 per cent. of the galactose oxidized. Raffinose yields under similar treatment, about 23 per cent. of mucic acid, which proves that the galactose group is contained in that sugar. Raffinose, therefore, is composed of one molecule each of dextrose, levulose and galactose.

213. Ammonium Molybdate Test.—The color reaction produced with ammonium molybdate and acetic acid is the only one which can be said to be characteristic of levulose, yet it is not wholly for levulose, as other sugars give the color but less rapidly and less extensively. The test is carried on as follows:

Ten cubic centimeters of a four per cent. ammonium molybdate solution, 0.2 gram of glacial acetic acid are added to the sugar solution, and heat the mixture in water bath at 95-98°. After three minutes a deep blue coloration appears, owing to the reduction of the molybdic acid, if fructose is present.

In the test, no other free acids either organic or inorganic than acetic should be present. If present, they may be removed by adding lead acetate to the solution, precipitating the excess lead with sodium carbonate, and acidify the filtrate with acetic acid.

214. Properties of the Principal Sugars.—In the space that can be devoted to this portion of the subject, it can only be hoped to make a few remarks on the principal sugars that occur in agricultural products, taking the individual sugars, giving their main

sources, their physical and chemical properties and their estimation.

Under monosaccharids will be mentioned arabinose, xylose, rhamnose, pentosans and methyl pentosans, dextrose, manose, galactose, levulose and sorbinose; under disaccharids, sucrose, maltose, lactose, melibiose; under trisaccharids, raffinose, and finally the tetra-saccharid, stachyose.

Arabinose $C_5H_{10}O_5$. This is sometimes called pektion sugar. It is an aldose and the usual form is *l*-arabinose, and *d* and *i* modifications at present are of only theoretical interest.

Arabinose does not occur free in nature, but is widely distributed in the vegetable kingdom as a polysaccharid of high molecular weight, araban. It occurs principally in the vegetable gums of the peach and cherry trees. From these, with long hydrolysis with acid, the sugar can be obtained. It is easily soluble in hot and cold water, less soluble in 90 per cent. alcohol and insoluble in absolute alcohol and ether. From its water solution, it does not crystallize easily. It shows mutarotation. A freshly prepared 10 per cent. arabinose solution, according to Lippman, showed $\alpha_D = + 150.50$ and after 24 hours $+ 105^\circ$. The specific rotation changes with concentration and temperature. In concentration (p) of 5 to 20 the $\alpha_D^t = + 108.189 - 0.3962 p + 0.01389 p^2$. For temperature (t) of from 5° to 20° , the equation becomes $\alpha_D = + 108.189 - 0.3962 p + 0.01389 p^2 + (20 - t) 0.3$. Its natural form is the mono-hydrate. It is not fermentable, but is acted upon by certain bacteria and forms compounds with acetic, nitric, and benzoic acids, and with some bases. With phenylhydrazin it forms a hydrazone and osazons, and according to Neuberg²⁶ diphenylhydrazin precipitates it quantitatively.

In a pure state in solution, it can be estimated by Allihn's Fehling method using the factor of Browne. It is estimated along with the other pentosans and pentose sugars by distillation with hydrochloric acid in the form of furfural. Pure araban is said to have the formula $C_{10}H_{16}O_4$ and is a white gummy mass showing a rotation of $\alpha_D = - 123^\circ$.

²⁶ Berichte der Deutschen chemischen Gesellschaft, 1900, 33 : 2254.

Xylose ($C_5H_{10}O_5$ — *Xylan* ($C_6H_8O_4$). This is sometimes called "wood sugar. Like arabinose, it is an aldose and the common form is *l*-xylose.

It does not occur free in nature, but is very widely distributed in the vegetable kingdom, in the polysaccharid form called xylan or "wood gum." In this form it occurs in various woods in percentages up to 20 per cent., figured to the ash-free dry basis. In straws of all kinds the percentage goes up to 40. In stalks of Indian corn, I have also found as high as 40 per cent.³⁷ From these materials, xylan can be extracted by treatment with alkalis or lime-milk, and from this liquor precipitated by alcohol.³⁸ In a pure state, xylan is a fine white porous, non-hydroscopic powder. Freshly prepared, it is soluble in hot water to a slight extent, but gelatinizes with cold water. In alkaline solutions, its rotation is strongly levo-rotatory, and is variously given from $a_D = -96.55$ to $a_D = -69.92$ as prepared from various sources. In alkaline solutions, it is precipitated by alcohol, lime and basic lead acetate. On heating with acids, even dilute, xylan is hydrolyzed to the sugar xylose.

Xylose has a sweet taste, is soluble in water and hot alcohol, but not in cold, absolute alcohol or ether. From its water solution, it is fairly easily crystallized.

A freshly prepared solution shows muta-rotation. According to Tollens a solution of 2.21398 grams in 20 cubic centimeters shows $a_D = +76.61$ at first, dropping to a constant after $2\frac{1}{2}$ hours of $+19.22$. Concentration and temperature change the specific rotation. If the concentration is represented by p the increase in specific rotation with increasing concentration is represented by the formula $a_D = 18.095 + 0.06986p$. For concentrations above 34.3% $a_D = 23.089 - 0.1827p + 0.00312p^2$. As the temperature rises, the specific rotation increases, for example, at $15^\circ a_D = +18.889$; at $20^\circ = +18.909$; at $25^\circ = +19.248$ and at $30^\circ = +19.628$.

Xylose is not fermentable, but is acted upon by bacteria. It

³⁷ Wiley, Bulletin de l'Association des Chimistes de Sucrerie et de Distillerie, 1899, 16: 1212.

³⁸ Stone, J. Amer. Chem. Soc., 1894, 16: 731.

forms compounds with nitric and acetic acids, etc., and with some bases. When converted to xylonic acid and treated with cadmium carbonate and bromin, a characteristic double salt is prepared.³⁹

With phenylhydrazin, both a hydrazone and an osazon are formed.

In a pure state its estimation can be accomplished by Allihn's method using Browne's factor, or by the distillation method to be described later. The latter method allows of its estimation in mixtures. Some success has been met in its separation from other sugars by means of the cadmium compound spoken of above.

Rhamnose ($C_6H_{12}O_6$). This has been known under the name of isodulcitol or rhamnodulcitol. It belongs to a class of carbohydrates, methylpentoses, of which other representatives have been latterly discovered in plants. In these, one of the hydrogen atoms in the primary alcohol group of pentose is replaced by the methyl group. It shows many of the characteristic reactions of glucose.

It does not occur free in nature, but in the form of a glucoside exists in many plants. The principal glucosides containing it are quercitrin and xanthorhamnin. It also occurs in some leaves and berries. From these glucosides, it is prepared by heating with dilute sulfuric acid, saturating the filtrate with pure barium carbonate and allowing crystallization to take place after concentration in vacuum.

It crystallizes with a molecule of water, the hydrate having the formula $C_6H_{14}O_6$. Rhamnose is soluble in hot and cold water, in 90 per cent. cold alcohol much less soluble, and only very slightly soluble in absolute alcohol and ether.

Rhamnose solutions show muta-rotation. A solution of a hydrate two minutes after preparing showed $\alpha_D - 5^\circ$ and became constant at $+ 8.56^\circ$. Up to 40 per cent. solution, the rotation does not change appreciably with concentration. Temper-

³⁹ Tollens, *Berichte der Chemischen Gesellschaft*, 1900, **33**: 132.

ature, however, affects the rotation. A formula given by *Gernez** for this is:

$$\alpha_D = 9^{\circ}.22 - 0.03642t + 0.0000123 t^2.$$

The hydrate begins breaking up a little below 100° , and at from 105° to 110° , one molecule of water is given off and the anhydrid is formed. In this latter form it is an amorphous, glassy, hygroscopic mass. The anhydrid has been prepared in a crystalline form.

Rhamnose is not fermentable, but is acted upon by bacteria. It forms compounds with acetic, nitric acid, etc., and with some bases. With phenylhydrazin it forms both hydrazins and osazons that are crystalline in structure.

It reduces Fehling solution, but no tables have so far been prepared for it. 10 cubic centimeters of Fehling solution equals 0.0522 gram of rhamnose according to Will.⁴⁰ Hence its estimation in water solutions in a free state can be made that way. It is best estimated by distillation with hydrochlorid acid as methylfurfurol.

Pentosans and Methyl-Pentosans. As has been stated the pentoses and methyl pentoses do not occur free in the vegetable world; but in the form of ligneous compounds of glucosides. As in the analysis of a plant, we estimate the pentosans and methyl-pentosans by the method of distillation with hydrochloric acid and determining the quantity of furfurol produced.

DETERMINATION OF STARCH.

215. Constitution of Starch.—The molecule of starch is without doubt formed by the condensation of a large number of hexose bodies. On account of its great insolubility its molecular weight has not been determined with any degree of accuracy. Its formula may be expressed either as $(C_6H_{10}O_5)_n$ or $(C_{12}H_{20}O_{10})_n$. It is insoluble in cold water and other common solvents and does not pass into solution in any reagent without undergoing a change of structure. In hot water it forms a paste and when heated under pressure with water it undergoes a partial change and be-

* *Comptes Rendus*, 121 : 1150.

⁴⁰ *Berichte der Chemischen Gesellschaft*, 1885, 18 : 1311.

comes soluble. Heated with acids or subjected to the action of certain ferments it suffers hydrolysis and is transformed into dextrin, maltose and dextrose. In analytical work an attempt is usually made to transform the starch entirely into dextrose, the quantity of which is then determined by some of the processes already given. All starches possess the property of giving an intensely blue color with iodine and this reaction serves to detect the most minute quantity of the material.

Starch grains derived from different sources are distinguished by differences in size and appearance. In most cases a careful examination of the starch particles will reveal their origin. The greatest part of the cereal grains is composed of starch, the percentage ranging from sixty to eighty. Rice has the greatest percentage of starch in its composition of any substance. Certain root crops are also rich in starch, such as the potato, artichoke and cassava. Starch appears as one of the first products of vegetable metabolism, according to some authorities, preceding the formation of sugars. By reason of its greater complexity, however, it is more probable that the production of simple sugars precedes the formation of the more complex molecule. Starch granules are probably used as a food by the plant in the building of more complex structures and the excess of this food is stored in the seeds and in tubers.

216. Separation of Starch Particles.—Advantage is taken of the small size of the starch particles to secure their separation from the other vegetable structures with which they are associated. The substances containing starch are reduced to a pulp as fine as possible, and this pulp being placed on a fine cloth the starch particles are washed through the cloth with water. The milky filtrate carrying the starch is collected in an appropriate holder and, after some time, the particles subside. They may then be collected and dried. While this process is the one used commercially in the manufacture of starch, it can only give approximate data respecting the actual quantity of starch in a given weight of the sample. It is not quite possible by this method to get all the starch separated from the rest of the

vegetable matter, and particles of foreign substances, such as cellulose and albuminoid matters, may pass through the filter cloth and be found with the deposited granules. It follows from this that the quantitative determination of the starch in a given sample by any direct method is only approximately exact.

217. Methods of Separation.—Hot acids cannot be safely employed to dissolve starch from its natural concomitants because other carbohydrates bodies become soluble under similar conditions. In such cases the natural sugars which are present should be removed by cold water and the starch dissolved from the residue by a diastatic ferment. Instead of this the sugars may be determined in a separate portion of the pulped material and the starch, together with the sugars, determined, and the quantity of sugar found deducted from the final result.

The starch, after separation with diastase, is converted into dextrose by one of the methods to be given and the resulting dextrose determined by one of the approved methods.

218. Separation with Diastase.—Diastase or malt extract at a temperature of about 65° rapidly renders starch soluble. Cereals, potato meal and other starch-holding bodies are dried, first at a low temperature, and extracted with ether or petroleum to remove fat. The material is then rubbed up with water, boiled, cooled to 65°, and treated with malt extract (diastase) prepared as given below. One kilogram of ground green malt is mixed with one liter of glycerol and an equal quantity of water, and allowed to stand, with frequent shaking, for eight days. After that time the mixture is filtered, first through a small filter press and afterwards through paper. In case no filter press is at hand the mixture may be pressed in a bag and the liquor obtained, filtered. Malt extract obtained in this way will keep its diastatic properties for a long time. In its use, blank determinations must be made of the dextrose produced by treating portions of it with hydrochloric acid. For three grams of starchy material 25 cubic centimeters of the malt solution should be used and the mixture kept at 65° for two hours.⁴¹

⁴¹ Zeitschrift für Physiologische Chemie, Band 12, Ss. 75-78.

219. Method in Use at the Halle Station.—The method of separating starch from cereals, potatoes and other starch-holding materials, employed at the Halle station, is essentially the same as already described.¹²

The malt extract used is prepared immediately beforehand, inasmuch as no preservative is added to it. It can be quickly prepared by digesting, for a short time at not above 50°, 100 grams of finely ground dried malt with one liter of water and separating the extract by filtration. This extract will keep only a few hours.

The material in which the starch is to be determined is dried and extracted with ether. From two to four grams of the extracted material, according to the amount of starch which it contains, are boiled for half an hour with 100 cubic centimeters of water, cooled to 65°, treated with ten cubic centimeters of malt extract and kept at the temperature named for half an hour. It is then again boiled for fifteen minutes, cooled to the temperature mentioned and again treated with malt extract as above. Two treatments with malt extract are usually sufficient to bring all the starch into solution. Finally it is again boiled and the volume completed to 250 cubic centimeters and thrown upon a filter. Two hundred cubic centimeters of the filtrate are converted into dextrose by boiling with hydrochloric acid, and the rest of the analysis is conducted in the usual manner. The dextrose value of the quantity of malt extract used must be determined upon a separate portion thereof, and the quantity of dextrose found deducted from the total amount obtained in the analysis.

220. Principles of the Methods of Determination.—In the approximately pure state in which starch exists in the trade, it may be determined by conversion into dextrose and estimating the latter by one of the methods given. It is probable that there is no known method by which starch can be entirely converted into dextrose, and all the methods of hydrolysis, when used for quantitative purposes, must be standardized, not by the theoretical

¹² Maercker-Delbrück, *Handbuck der Spiritusfabrikation*, 1908, 9th Edition : 159.

quantity of dextrose which a given weight of pure starch should yield, but by the actual quantity obtained. Starch is not largely converted into dextrose by any of the diastatic ferments which produce principally maltose and dextrans. Recourse must therefore be had to strong acids. In practice, hydrochloric is the one usually employed.

221. Estimation of Water.—In prepared or commercial starches the water may be determined by heating in a partial vacuum. The temperature at first should be low, not exceeding 60° . After drying for an hour at that heat the temperature may be gradually increased. The last traces of water come off from starch with difficulty, and the final temperature may be carried a little beyond 100° without danger of decomposition.

Ost recommends the use of an atmosphere of hydrogen or illuminating gas.⁴³ One and a half grams of the finely powdered sample are placed in a drying tube of convenient form and heated in a stream of dry hydrogen. The temperature at first is kept at about 60° for several hours and is then gradually increased to 120° . Ost states that even at 150° the sample preserves its pure white color, but so high a temperature is not necessary. Maercker, at the Halle station, makes use of the same process, but employs illuminating gas instead of hydrogen. The importance of beginning the desiccation at a low temperature arises from the fact that at a higher temperature, before the greater part of the water is driven off, the starch will suffer a partial fusion and form a paste which is very difficult to dry. The dried sample must be kept in a stoppered vessel to prevent the absorption of hygroscopic moisture.

222. Estimation of Ash.—When the drying is accomplished in a flat platinum dish, the same sample may serve for incineration. Otherwise the incineration may be accomplished in another portion of the sample by following directions already given.⁴⁴

⁴³ Chemiker-Zeitung, 1895, 19 : 1501.

⁴⁴ Paragraphs 28-32, this volume.

223. Nitrogen.—Even very pure samples of starch may contain a little nitrogen which is most conveniently determined by most combustion.⁴⁵

As a rule, in commercial starches of good quality, the quantity of pure starch may be considered to be the remainder after subtracting the sum of the weights of water, ash and nitrogen multiplied by 6.25, from the original weight of the sample taken.

Example:—

Per cent of moisture found.....	12.85
" " " ash found	0.08
" " " nitrogen \times 6.25	0.27
Sum.....	13.20
Per cent of pure starch in sample	86.80

Samples of starch usually contain also traces of fat and fiber, and these when present in weighable quantities, should be determined and proper deductions made.

224. Polarization of Starch.—Starch may be prepared for polarization by dissolving it in cold hydrochloric acid. The process as carried out by Effront is as follows.⁴⁶ Five grams of starch are rubbed with 20 cubic centimeters of cold concentrated hydrochloric acid for nearly 10 minutes or until the solution is quite clear. The volume is completed to 200 cubic centimeters with water and the solution polarized. By this process there is always produced a notable quantity of reducing sugars, and for this reason it must be admitted that a portion of the starch has suffered complete hydrolysis. Ost therefore recommends the use of an acid of 1.17 specific gravity, and the gyrodyn of the soluble starch thus produced is found to vary from $[\alpha]_D = 196^\circ.3$ to $196^\circ.7$. When acid of 1.20 specific gravity is employed the gyrodyn falls to $[\alpha]_D = 194.2$.⁴⁷ For approximately correct work the solution with the weaker hydrochloric acid and subsequent polarization is to be recommended as the most rapid method for starch determination.

⁴⁵ Principles and practice of Agricultural Analysis, 1908, 2 : 346.

⁴⁶ Chemiker-Zeitung, 1895, 19 : 1502.

Moniteur Scientifique, 1887, 29 : 538.

⁴⁷ Chemiker-Zeitung, 1895, 19 : 1502.

225. Solutions of Starch at High Pressure.—Starch may also be brought into a condition suited to polarization by dissolving in water at a high temperature and pressure. The solution is accomplished in an autoclave.

From two to three grams of starch are used and from 80 to 90 cubic centimeters of water. The starch is first reduced to a pasty state by heating with the water and, when evenly distributed throughout the flask, is rendered soluble by heating from three to five hours in an autoclave at from two to three atmospheres. The material is entirely without action on an alkaline copper solution. After heating, the volume of the solution is completed to 100 cubic centimeters and it is then polarized. The gyrodyn of starch dissolved in this way varies from $[a]_D = 196^\circ.5$ to $197^\circ.6$.

Starch is prepared by Baudry for polarization by boiling with salicylic acid.⁴⁹ The gyrodyn of starch dissolved in this way is $[a]_D = 200^\circ.25$.

226. Polarization after Solution in Dilute Nitric Acid.—Guichard recommends saccharification with 10 per cent. nitric acid (10 cubic centimeters strong acid, 90 cubic centimeters water).⁵⁰ This treatment, even after prolonged boiling, gives only a light straw color to the solution which does not interfere with its polarization.

In working on cereals four grams of the finely ground material, in which the bran and flour are intimately mixed, are used.

The material is placed in a flask of about 500 cubic centimeters capacity, with 100 cubic centimeters of the dilute acid. The flask is closed with a stopper carrying a reflux condenser. After boiling for an hour the contents of the flask are filtered and examined in the saccharimeter. The dextrose formed is determined by the polarimetric data and the quantity of starch transformed calculated from the dextrose. The following formula is used:

$$A = \frac{av \times 25 \times 0.016}{2 \times 52.8}.$$

⁴⁸ Chemiker-Zeitung, 1895, 19 : 1502.

⁴⁹ Jahresberichte des Agrikultur-Chemie, neue folge, 1892, 15 : 661.

⁵⁰ Journal de Pharmacie et de Chimie, 5^e serie, 1892, 25 : 394.

In this formula a = the rotation in angular degrees, v = the volume of the liquid and A = the starch transformed.

In this method no account is taken of the sucrose and other sugars which are present in cereals. In the case of sucrose the left-handed sugar produced by treatment with nitric acid would diminish the rotation to the right and thus introduce an error. On the other hand the dextrose formed from the fiber of the bran would be calculated as starch. If these two errors should be compensating the method might prove practical.

227. Rapid Estimation of Starch.—For the rapid estimation of starch in cereals, cattle foods and brewery refuse, Hibbard recommends a method which is carried out as follows:

The malt extract is prepared by covering ground, dry malt with water containing from 15 to 20 per cent. of alcohol. The object of adding alcohol is to preserve the filtered extract. It exercises a slight retarding effect on the action of the diastase, but prevents the malt extract from fermenting. After standing for a few hours in contact with the malt, the liquid is separated by filtration and is then ready for use. The substance in which the starch is to be determined should be dry enough to be finely pulverized, but previous extraction with ether is omitted. Enough of the material to contain at least half a gram of starch is placed in a flask with 50 cubic centimeters of water and from one to two cubic centimeters of malt extract added. The mixture is at once heated to boiling with frequent shaking to prevent the formation of clots. The addition of the diastase before boiling is to aid in preventing the formation of lumps. After boiling a minute the mixture is cooled to 60° and from two to three cubic centimeters of the malt extract added. It is then slowly heated until it again boils, consuming about 15 minutes, when, after cooling, it is tested with iodine for starch. If a blue color be produced the operation above described is repeated until it fails to reappear. The mixture is then made up to a standard volume, thrown on a linen filter and an aliquot part of the filtrate, representing from 200 to 300 milligrams of starch, is boiled with five cubic centimeters of hydrochloric acid, of 30 per cent. strength, for half an hour. The

total volume of the liquid before boiling should be completed to 60 cubic centimeters. By the method above described, it is claimed that the determination of starch in a cereal or similar substance can be completed within two hours. The chief amount of time saved is in the heating with the malt extract, which instead of being continued for two hours, as usually directed, can be accomplished in 30 minutes.⁵¹

228. Precipitation of Starch with Barium Hydroxid.—The tendency of carbohydrate bodies to unite with the earthy bases has been utilized by Asboth as a basis for the quantitative determination of starch.⁵²

About three grams of the finely ground sample containing the starch, or one gram of pure starch, are rubbed up in a mortar with water and the detached starch remaining suspended in the wash water is poured off. This operation is repeated until all the starch is removed. In difficult cases hot water may be used. The starch thus separated is heated in a quarter liter flask to the boiling point to reduce it to the condition of paste. When the paste is cold it is treated with 50 cubic centimeters of the barium hydroxid solution, the flask closed and well shaken for two minutes. The volume is then completed to the mark with 45 per cent. alcohol, the flask well shaken and allowed to stand. In a short time the barium-starch compound separates and settles. Fifty cubic centimeters of the clear supernatant liquor are removed with a pipette, or the liquor may be passed through a filter and the quantity mentioned removed for titration of the residual barium hydroxid after the addition of a few drops of phenolphthalein solution.

The quantity of barium hydroxid remaining, deducted from the original quantity, gives the amount which has entered into composition with the starch; the composition of the molecule being $\text{BaOC}_2\text{H}_{40}\text{O}_{20}$, which contains 19.10 per cent. of barium oxid and 80.90 per cent. of starch.

The set solution of barium hydroxid must be preserved from

⁵¹ Journal of the American Chemical Society, 1895, **17** : 64.

⁵² Repertorium der Analytischen Chemie, 1887, **7** : 299.

contact with the carbon dioxid of the air. The burette should be directly attached to the bottle holding the set solution, by any of the usual appliances, and the air entering the bottle must be deprived of carbon dioxid. The water used in the work must be also free of air, and this is secured by boiling immediately before use.

Example.—A sample of flour selected for the analysis weighed 3.212 grams. The starch was separated and reduced to paste in the manner described above. Thirty and four-tenths cubic centimeters of tenth-normal hydrochloric acid were exactly neutralized by 10 cubic centimeters of the barium hydroxid solution. After treatment as above described, 50 cubic centimeters of the clear liquor, corresponding to 10 cubic centimeters of the added barium hydroxid, required 19.05 cubic centimeters of tenth-normal hydrochloric acid. Then $30.4 - 19.05 = 11.35$, and $11.35 \times 5 = 56.75$, which number corresponds to the total titration of the residual barium hydroxid in terms of tenth-normal hydrochloric acid. This number multiplied by 0.0324, *viz.*, starch corresponding to one equivalent of barium, gave 1.8387 grams of starch or 57.24 per cent. of the weight of flour employed.

The barium hydroxid method is unsatisfactory when applied to cereals. The principle of the process, however, appears to be sound, and with a proper variation of working details, it may become practical.

229. Fixation of Iodin.—In addition to forming a distinctive blue color with iodine, starches have the power of fixing considerable quantities of that substance. The starches of the cereals have this power in a higher degree than those derived from potatoes. In presence of a large excess of iodine the starches of rice and wheat have a maximum iodine-fixing power of about 19 per cent. of their weight. When only enough of iodine is employed to enter into combination the percentage absorbed varies from nine to 15 per cent. The absorption of iodine by starches is a matter of importance from a general chemical standpoint, but as at present determined has but little analytical value. It is evident, however, that this absorption must take place according to definite

chemical quantities and the researches of investigators may in the future discover some definite quantitative method of measuring it.⁵³

230. Identification of Starches of Different Origin.—It is often important, especially in cases of suspected adulteration, to determine the origin of the starch granules. For this purpose in the absence of any history of the sample the microscope is the chief resort. In many cases it is easy to determine the origin of the starch by the size or the shape and marking of the grains. In mixtures of more than one kind of starch the distinguishing features of the several starches can be clearly made out in most instances. There are, however, many instances where it is difficult to discriminate by reason of the fact that the characteristics of starch granules vary even in the same substance and from year to year with varying conditions of culture.

In many cases the illustrations of the forms and characteristics of starch granules which are found in books are misleading and little reliance can be placed on any illustrations which are not either photographs or drawings made directly from them. In the microscopic study of starches the analyst will be greatly helped by the following descriptions of the characteristic appearance of the granules and the classifications based thereon.⁵⁴

231. Vogel's Table of the Different Starches and Arrowroots of Commerce.—The measurements in all cases are in micromillimeters, (μ) = .001 millimeter.

A. Granules simple, bounded by rounded surfaces.

I. Nucleus, central, layers concentric.

a. Mostly round, or from the side, lens-shaped.

1. Large granules 39.6-52.8 μ , *rye starch*:

2. Large granules 35.2-39.6 μ , *wheat starch*:

3. Large granules 26.4 μ , *barley starch*.

b. Egg-shaped, oval, kidney-shaped: Hilum often long and ragged:

1. Large granules 32-79 μ , *leguminous starches*.

⁵³ Rouvier, *comptes rendus*, 1892, **114**: 1366; 1893, **117**: 461; 1894, **118**: 743; 1895, **120**: 1179.

⁵⁴ Division of Chemistry, Bulletin 13, 1887: 154 et seq.

II. Nucleus eccentric, layers plainly eccentric or meniscus-shaped.

a. Granules not at all or only slightly flattened:

1. Nucleus mostly at the smaller end; 60-100 μ , *potato starch*:

2. Nucleus mostly at the broader end or towards the middle in simple granules; 22-60 μ , *maranta starch*.

b. Granules more or less strongly flattened.

1. Many drawn out to a short point at one end.

a. At most 60 μ long, *curcuma starch*:

b. As much as 132 μ long, *canna starch*:

2. Many lengthened to bean-shaped, disk-shaped, or flattened; nucleus near the broader end; 44-75 μ , *banana starch*:

3. Many strongly kidney-shaped; nucleus near the edge; 48-56 μ , *sisyrinchium starch*:

4. Egg-shaped; at one end reduced to a wedge, at the other enlarged; nucleus at smaller end; 50-70 μ *yam starch*:

B. Granules simple or compound, single granules or parts of granules, either bounded entirely by plain surfaces, many-angled, or by partly round surfaces.

I. Granules entirely angular.

1. Many with prominent nucleus: At most 6.6 μ , *rice starch*:

2. Without a nucleus: The largest 8.8 μ , *millet starch*:

II. Among the many-angled also rounded forms.

a. No drum-shaped forms present, angular form predominating.

1. Without nucleus or depression very small; 4.4 μ , *oat starch*:

2. With nucleus or depression; 13.2-2.2 μ .

a. Nucleus or its depression considerably rounded; here and there the granules united into differently formed groups; *buckwheat starch*:

- b.* Nucleus mostly radiate or star-shaped; all the granules free; *maize (corn) starch*:
 - b.* More or less numerous kettledrum and sugar-loaf like forms.
 - 1. Very numerous eccentric layers; the largest granules 22-35.2 μ , *batata* (sweet potato) *starch*:
 - 2. Without rings; 0.008-0.022 mm.
 - a.* In the kettledrum-shaped granules the nuclear depression mostly widened on the flattened side; 8-22 μ , *cassava starch*:
 - b.* Depression wanting or not enlarged.
 - aa.* Nucleus small, eccentric; 8-16 μ , *pachyrhizus starch*:
 - bb.* Nucleus small, central, or wanting.
 - aaa.* Many irregular angular forms; 8-17.6 μ *sechium starch*:
 - bbb.* But few angular forms; some with radiate, nucleal fissure; 8-17.6 μ , *chestnut starch*.

C. Granules simple and compound; predominant forms, oval, with eccentric nucleus and numerous layers; the compound granule made up of a large granule and one or more relatively small kettledrum-shaped ones; 25-66 μ , *sago starch*.

232. Preparation of Starches for Microscopical Examination.--

The approximately pure starches of commerce may be prepared for microscopic examination by rubbing them up with water and mounting some of the suspended particles by one of the methods to be described below.

In grains, seeds and nuts the starch is separated by grinding with water and working through bolting cloth. The starch which is worked through is allowed to subside, again beaten up with water if necessary and the process continued until the grains are separated sufficiently for microscopic examination. A little potash or soda lye may be used, if necessary, to separate the granules from albuminous and other adhering matter. The analyst should have a collection of samples of all common starches of known origin for purposes of comparison.

The granules are mounted for examination by plain light in a medium of water.⁵⁵

233. Appearance in Balsam with Polarized Light.—When mounted in Canada balsam which is often used instead of water the starches are scarcely visible under any form of illumination with ordinary light, the index of refraction of the granules and the balsam being so nearly alike. When, however, polarized light is used the effect is a striking one. It is very easy to distinguish all the characteristics, except the rings, the center of the cross being at the nucleus of the granule.

With the selenite plate a play of colors is produced, which is peculiar to some of the starches, but is of little value for discriminating purposes. There is no one feature in which published descriptions of the starches are more at variance than in their behavior with polarized light. This is due in part at least to the difference in efficiency of different micropolarizers, some producing much better results than others. The activity varies in intensity with the size of the grains. Thus a small potato granule does not polarize as vigorously as the large grains, while on the other hand large granules of some starches will not be as active as the small granules of some others. The utility of polarized light in the examination of starch grains is not as great as has usually been believed.

234. Description of Typical Starches.—The more commonly occurring starches are described by Richardson as they appear under the microscope magnified about 350 diameters.⁵⁶

Maranta Starch.—Of the same type as the potato starch are many of the arrowroots, the only one of which commonly met with in this country being the Bermuda, the starch of the rhizome of *Maranta arundinacea*, and the starch of turmeric.

The granules are usually not so varied in size or shape as those of the potato, averaging about 0.07 millimeter in length as may be seen in Fig. 47. They are about the same size as the average of the potato, but are not often found with the same maximum or

⁵⁵ Division of Chemistry, Bulletin 13, 1887 : 158.

⁵⁶ Division of Chemistry, Bulletin 13, 1887 : 158 et seq.



Fig. 47.—Maranta Starch. x 350.



Fig. 48.—Potato starch. x 350.



Fig. 49.—Ginger Starch. x 350.



Fig. 50.—Sago Starch. x 350.



Fig. 51.—Pea Starch. x 350.



Fig. 52.—Bean Starch. x 350.



Fig. 53. — Wheat Starch. x 350.

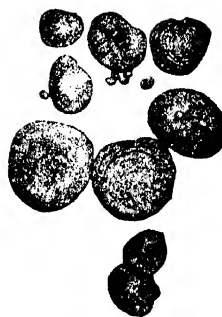


Fig. 54. — Barley Starch. x 350.



Fig. 55. — Rye Starch. x 350.



Fig. 56. — Oat Starch. x 350.



Fig. 57. Indian Corn Starch. x 350.



Fig. 58. Rice Starch x 350.

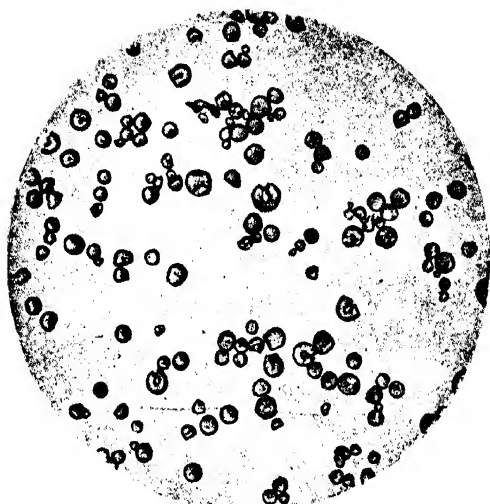


Fig. 59. Cassava Starch. x 150. Plain illumination.

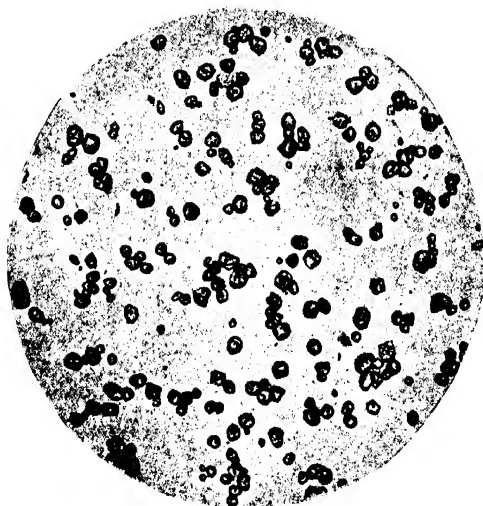


Fig. 60. Indian Corn Starch. x 150. Plain illumination.

minimum magnitude, which circumstance, together with the fact that the end at which the nucleus appears is broader in the maranta and more pointed in the potato, enables one to distinguish the two starches without difficulty. With polarized light the results are similar to those seen with potato starch, and this is a ready means of distinguishing the two varieties, by displaying in a striking way the form of the granule and position of the hilum.

Potato Starch.—The starch grains of the potato are very variable in size, being found from 0.05 even much less than this not infrequently only 0.025 mm. to 0.110 millimeter in length, and in shape from oval and allied forms to irregular and even round in the smallest. These variations are illustrated in Fig. 48, but the frequency of the smaller granules is not as evident as in some other cases. The layers are visible in some granules with great distinctness and in others hardly at all, being rather more prominent in the starch as obtained from a freshly cut surface. The rings are more distinct, too, near the hilum or nucleus, which in this, as in all tuberous starches, is eccentric, shading off toward the broader or more expanded portion of the granule. The hilum appears as a shadowy depression, and with polarized light its position is well marked by the junction of the arms of the cross. With polarized light and a selenite plate a beautiful play of colors is obtained. The smaller granules, which are nearly round, may readily be confused with other starches, but their presence serves at once to distinguish this from maranta or Bermuda arrowroot starch. Rarely compound granules are found composed of two or three single ones each with its own nucleus.

Ginger Starch.—This starch (from *Zingiber officinale* Pascoe,) is of the same class as those from the potato and maranta and several others which are of underground origin. In outline the granules are not oval like those named, but more rectangular, having more obtuse angles in the larger ones, having a small point at the hilum and of the grain and being cylindrical or circular in outline in the smaller, as indicated in Fig. 49. They measure

mostly from 0.020 mm to 0.035 mm in length, both in size and form. The rings are plainly visible but appear only as cords of circles.

Sago Starch.—This exists in two modifications in the market; as raw and as prepared sago. In the prepared condition it is characterized by a larger circular depression in the center of most of the granules due to the cooking, but in addition numerous granules are usually present which show the characteristic form with the truncated granules. The rings are not visible. They are mostly circular in form or approaching it, and vary from 0.025 to 0.065 millimeter in diameter, as indicated in Fig. 50.

Pea and Bean Starches.—Under anything like fair lighting conditions with a good instrument they are fully if not more visible than in maranta starch. The rings are scarcely visible, and the hilum is stellate or much cracked along a median line, the bean more so than the pea, the latter resembling fresh dough kneaded again into the center as in making rolls, and the former the shape assumed by the same after baking. The grains of both are somewhat variable in size, ranging from 0.025 to 0.070 millimeter in length, as shown in Figs. 51 and 52.

Wheat starch grains are quite variable in size, the larger grains measuring 0.020 to 0.035 millimeter in diameter. They belong to the same class as barley and rye, the hilum being invisible in the most of the grain and the rings not prominent. The granules are circular disks in form, and there are now and then contorted depressions resembling those in pea starch. They are the least regular of the three starches named and do not polarize actively. The typical forms of these granules are shown in Fig. 53.

Barley Starch is quite similar to that of wheat, but the grains do not vary so much in size, the most of them measure 0.018 to 0.025 millimeter, while some are as large as 0.030 millimeter. They have rings which are much more distinct, and very small granules adhering to the largest in bud-like forms, as seen in Fig. 54.

Rye Starch is more variable in size, many of the granules not exceeding 0.02 millimeter, while the largest reach 50 μ (0.050

millimeter). Its most characteristic features are the large size for some of the grains than for wheat and barley and the stellate hilum which is quite frequent and almost absent in the other kinds mentioned, and is the most simple in form of all the starches. Fig. 55 shows the appearance of the granules under the microscope.

Oat Starch is unique, being composed of large compound masses of polyhedral granules from 0.12 to 0.02 millimeter in length, the single granules rarely exceed 10 μ millimeter size, and many are less than this. Oat starch is also characterized by the occasional presence of spindle shaped grains. It does not polarize actively, and displays neither rings nor hilum. The illustration, Fig. 56, shows its nature with accuracy.

Indian Corn Starch.—The granules of maize vary in size from 0.005 to 0.020 millimeter, the most of the grains measuring 0.012 to 0.018 millimeter. They are mostly circular in shape or rather polyhedral, with rounded angles, as shown in Figs. 57 and 60. They form very brilliant objects with polarized light, but with ordinary illumination show but the faintest sign of rings and a well-developed hilum, at times star-shaped, and at others more like a circular depression.

Rice Starch is similar in shape to that of maize, but is readily distinguished from it since the grains rarely exceed 0.010 millimeter in diameter, the most of them ranging from 0.005 to 0.008 millimeter. The grain, however, is distinguished chiefly by its polygonal form, and its well-defined angles, as indicated in Fig. 58. The hilum is very faint and usually not visible at all. Several granules are at times united.

Cassava Starch.—This variety of starch is obtained from the root of the sweet cassava, which grows in great profusion in Florida. It is compared with maize starch in Figs. 59 and 60. The grains vary from 15 μ to even as large as 35 μ though not many exceed 25 μ . They are nearly spherical in shape, a large proportion of the grains having one, occasionally two and rarely

three or four truncated surfaces. The cassava starch as found in pearl tapioca is in a more or less cooked condition.⁵⁷

These descriptions, it will be seen, do not agree entirely with those of some other authors, but they are based on a somewhat extensive experience.

There are peculiarities of size, shape and appearance of starch granules, which must be allowed for, and the necessity for every investigator to compare a starch which he is desirous of identifying with authentic specimens, must always be recognized.

Importance of Distinguishing between Different Starches.-- Not only is it interesting from the purely scientific viewpoint to distinguish the starch granules from one another, but also it is of economic and legal importance. Starches are used largely as fillers, as in the manufacture of baking powders, and as adulterants, as found in condiments and sausages. Where no starch naturally exists in a substance as is the case in sausage, the mere discovery of the starch without determining its origin is a sufficient evidence of adulteration. On the contrary, in some condiments which naturally contain starch the addition of other starches for fraudulent purposes is evidenced by the size, shape and other physical characteristics of the grains. For instance, if a sago biscuit is found to be made of wheat or other flour by microscopic examination of its granules, it would be convincing evidence of misbranding.

⁵⁷ Division of Chemistry; Bulletin 44, 1894 : 14.

PART THREE

PROCESSES FOR DETECTING AND DETERMINING SUGARS AND STARCHES AND OTHER CARBOHYDRATES IN CRUDE OR MANUFACTURED AGRICULTURAL PRODUCTS.

235. Introduction.—In the preceding part directions have been given for the estimation of sugars and starches in approximately pure forms. In the present part will be described the most approved methods of separating these bodies and other carbohydrates from crude agricultural products and for their chemical examination. In many respects the processes which in a small way are used for preparing samples for analysis are employed on a large scale for technical and manufacturing purposes. It is evident, however, that the following paragraphs must be confined strictly to the analytical side of the question inasmuch as anything more than mere references to technical processes would lead into wide digressions.

In the case of sugars the analyst is for the most part quite as much in need of reliable methods of extraction and preparation as of processes for analysis. With starches the matter is more simple and the chief methods of separating them for examination were necessarily described in the previous part.

Sugars in fresh plants exist almost entirely in solution. This is true of all the great sources of the sugar of commerce, *viz.*, the palm, the maple, the sugar beet and sugar cane. This statement is also true of fruits and the natural nectar of flowers. By natural or artificial drying the sugar may be reduced to the solid or semisolid state as in the cases of raisins and honey. In certain seeds, deficient in water, sugars may possibly exist in a solid state naturally, as may be the case with sucrose in the peanut and raffinose in cotton seed.

Starches on the other hand when soluble, are probably not true

starches, but they partake more or less of a dextrinoid nature. Fine starch particles occur abundantly in the juices of some plants, as for instance sorghum, where they are associated with sugar and can be obtained from the expressed juice by subsidence. But even in such a case it is not certain that the starch enters into the general circulation. It is more likely formed locally by biochemical condensation of its constituents. Starches in a soluble or semisoluble state are transported, as a rule, to the tubers or seeds of plants where they are accumulated in large quantities as a reserve food for future growth. For a study of the plant metabolism whereby starch is produced and for its histological and physiological properties the reader may consult the standard authorities on vegetable physiology.⁵⁸

236. Sugar in the Sap of Trees.—Many trees at certain seasons of the year, carry large quantities of sugar in their sap. Among these the maple and sugar palm are pre-eminent. The sap is secured by cutting a pocket into the side of the tree or by boring into it and allowing the sap to run into an appropriate receptacle through a spile. The content of sugar in the sap of the maple and palm varies greatly. In some cases it falls as low as one and a half and in others rises to as much as six or seven per cent.⁵⁹ The sugar of maple sap is pure sucrose, but towards the end of the flowing season the sap coming from old spouts undergoes changes of a viscous nature due to fermentation, or inversion, forming traces of invert sugar, but if the sap is fresh and free from contamination, no invert sugar is noted. This viscous condition of sap at end of season is due to bacterial action.⁶⁰ In this country the sap of the maple may flow freely on any warm day in winter, but the sugar season proper begins about February 15th in Southern Ohio and Indiana, and about March 25th in Vermont. It lasts from six weeks to two months. The sap flows best during moderately warm, still days, after a light freeze.

In addition to sugar the maple sap contains a trace of albumin.

⁵⁸ Vines, *Physiology of Plants*, 1886 : Nägeli, *Beiträge zur näheren Kenntniss der Stärkegruppe*, 1874.

⁵⁹ Division of Chemistry, Bulletin 5, 1885 : 191 et seq; New Hampshire Experiment Station, Bulletin 25, 1895.

⁶⁰ Edson, Vt. Exp. Station, Bull. 151.

noid matters and some malic acid combined with lime.⁶¹ As a rule it can be subjected to polarization without preliminary clarification

237. Determination of Sugar in Saps.—In most cases the sap may be directly polarized. Its specific gravity is obtained by a spindle or pyknometer, and the percentage of solids taken directly from the table, the degree brix corresponding to the sugar percentage.

To determine the quantity of sugar in the sap by polarization proceed as follows:

Multiply the specific gravity of the sap by 100 and divide the product by 26.048. Divide the direct reading of the sap on the sugar scale by the quotient obtained above, and the quotient thus obtained will be the correct percentage of sugar in the original solution.

The formula is applicable for those instruments in which 26.048 grams represent the normal quantity of sugar which in 100 cubic centimeters reads 100 divisions on the scale. When other factors are used they should be substituted for 26.048 in the above formula. For the new international standard polariscopes the factor is 26.

The principle of the calculation is based on the weight of the sap which is contained in 100 cubic centimeters, and this is evidently obtained by multiplying 100 by the specific gravity of the sap. Since 26.048 is the normal quantity of sugar in that volume of the solution the quotient of the actual weight divided by that factor shows how many times too great the observed polarization is. The simple division of the polariscopes reading by this factor gives the correct reading.

Example: Let the specific gravity of the sap be 1.015 and the observed polarization be 15.0. Then the true percentage of sugar in the sap is found by the equation:

$$x = 15 : \frac{101.5 \times 100}{26.048}$$

Whence $x = 4.00 =$ percentage of sugar in the sap.

⁶¹ Bureau of Chemistry, Bulletin 134, 1910.

The process outlined above is not applicable when a clarifying reagent such as lead subacetate or alumina cream must be used. But even in these cases it will not be found necessary to weigh the sap. A sugar flask graduated at 100 and 110 cubic centimeters is used and filled to the first mark with the sap, the specific gravity of which is known. The clarifying reagent is added, the volume completed to the second mark with water, and the contents of the flask well shaken and thrown on a dry filter. The observation tube, which should be 220 millimeters in length, is then filled with the clear filtrate and the rest of the process is as described above. A 200 millimeter tube may also be used in this case and the observed reading increased by one-tenth.

238. Estimation of Sugar in the Sap of Sugar Cane and Sorghum.—In bodies like sugar cane and sorghum the sap containing the

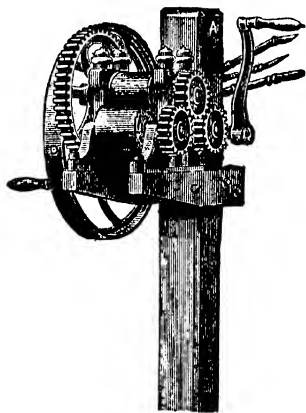


Fig. 61.—Laboratory Cane Mill.

sugar will not flow as in the cases of the maple and sugar palm. The simplest way of securing the sap of the bodies named is to subject them to pressure between rolls. A convenient method of obtaining the sap or juice is by passing the cane through a small three-roll mill indicated in the figure. Small canes, such as sorghum, may be milled one at a time, or even two or three when they are very small. In the case of large canes, it is necessary that

they be split and only half of them used at once. The mill should not be crowded by the feed in such a way as to endanger it or make it too difficult for the laborer to turn. From 50 to 60 per cent. of the weight of a cane in juice may be obtained by passing it through one of these small mills. Experience has shown that there is a little difference between the juice as first expressed and the residual sap remaining in the bagasse, but the juice first expressed may be used for analysis for control purposes as a fair representative of all that the cane contains.

To determine the percentage of juice expressed, the canes may be weighed before passing through the mill and the juice collected. Its weight divided by the weight of the original cane will give the per cent. of the juice expressed, calculated on the whole cane. Instead of weighing the juice the bagasse may also be collected and weighed; but on account of the rapidity with which it dries the operation should be accomplished without delay. The expressed juice is clarified with lead subacetate, filtered and polarized in the manner described in former paragraphs. Instead of weighing the juice, its specific gravity or degree brix may be taken by an accurate spindle and the volume of it, equivalent to a given weight, measured from a sucrose pipette.⁶²

A sucrose pipette has a graduation on the upper part of the stem which enables the operator to deliver double the normal weight for the polariscope used, after having determined the density of the juice by means of a spindle. A graduation of from 5° to 25° of the brix spindle will be sufficient for all variations in the density of the juice, or one covering a range of from 10° to 20° will suffice for most instances. The greater the density of the juice the less volume of it will be required for the weight mentioned. For general use, the sucrose pipette is graduated on the stem to deliver from 48 to 50.5 cubic centimeters, the graduations being in terms of the brix spindle. The graduation of the stem of this instrument is shown in the accompanying figure. In the use of the pipette it is only necessary to fill it to the degree on

⁶² Spencer, *Handbook for Cane-sugar Manufacturers*, 4th Edition, 1906: 121.

the stem corresponding to the degree brix found in the preliminary trial.

The quantities of juice corresponding to each degree and fractional degree of the brix spindle are given in the following table; calculated for the normal weight 26.048 grams for the ventzke and for 16.19 grams for the laurent scale. The measured

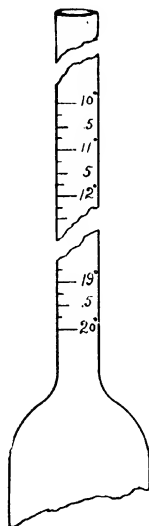


Fig. 62. —Weighing Pipette.

quantities of juice are placed in a 100 cubic centimeter sugar flask, treated with the proper quantity of lead subacetate, the volume completed to the mark, and the juice filtered and polarized in a 200 millimeter tube. The reading of the polariscope is divided by two for the factor 26.048 and by three for the factor 16.19.

In ordering sucrose pipettes the factor for which they are to be graduated should be stated.

TABLE FOR USE OF SUCROSE PIPETTES.

Degrees brix.	Cubic centimeters of juice for 26.048 factor. Divide reading by two.	Degrees brix.	Cubic centimeters of juice for 16.19 factor. Divide reading by three.
5.0	51.1	5.0	47.6
5.4	51.0	5.7	47.5
5.7	50.9	6.3	47.4
6.4	50.8	6.8	47.3
6.9	50.7	7.3	47.2
7.4	50.6	7.8	47.1
7.9	50.5	8.3	47.0
8.4	50.4	8.9	46.9
8.9	50.3	9.5	46.8
9.4	50.2	10.0	46.7
9.9	50.1	10.5	46.6
10.4	50.0	11.0	46.5
10.9	49.9	11.6	46.4
11.4	49.8	12.1	46.3
11.9	49.7	12.7	46.2
12.4	49.6	13.3	46.1
12.9	49.5	13.8	46.0
13.4	49.4	14.3	45.9
13.9	49.3	14.8	45.8
14.4	49.2	15.3	45.7
14.9	49.1	15.9	45.6
15.4	49.0	16.4	45.5
15.9	48.9	17.0	45.4
16.4	48.8	17.5	45.3
16.9	48.7	18.0	45.2
17.4	48.6	18.6	45.1
17.9	48.5	19.1	45.0
18.4	48.4	19.7	44.9
18.9	48.3	20.2	44.8
19.4	48.2		
19.9	48.1		

It is evident also that with the help of the foregoing table the measurements may be made by means of a burette. For instance, if the degree brix is found to be 19.9, 48.1 cubic centimeters are to be used. This quantity can be easily run from a burette. In order to make the pipette more convenient it has been customary to follow a method practiced by Carr, to attach a glass tube with a stopcock by means of a rubber tube to the upper part of the

pipette, whereby the exact level of the juice in the stem of the pipette can be easily set at any required mark.

In the polarization of dilute solutions, such as are found in the saps and juices referred to above, it must not be forgotten that the rotation of the sucrose is increased as the density of the solution is diminished. This change introduces a slight error into the work which is of no consequence from a technical point of view, but becomes a matter which must be considered in exact determinations. To avoid the annoyance of calculating the rotation for every degree of concentration, tables have been constructed by Schmitz and Crampton by means of which the actual percentage of sugar, corresponding to any degree of polarization, is determined by inspection. These tables may be used when rapid work is required.⁶³

239. Determination of Reducing Bodies in Cane Juices.—Sucrose in cane juices is constantly accompanied with reducing sugars, or other bodies which have a similar action on fehling liquor, which interfere to a considerable degree with the practical manufacture of sugar. It is important to determine with a moderate degree of accuracy the quantity of these bodies. For practical purposes, it may be assumed that their reducing power is the same as that of invert sugar and the percentage of these bodies present is calculated on that assumption. In the determination of these sugars or reducing bodies, the quantity weighed may be determined by an apparatus entirely similar to the sucrose pipette just described above. The quantity of juice used should be diluted as a rule to such a degree as not to contain more than one per cent. of the reducing bodies. For the best work, the juices should be clarified with lead acetate and the excess of lead removed with sodium carbonate. For technical control work in sugar factories, this process may be omitted as in such cases rapidity of work is a matter of considerable importance and the approximate estimation of the total quantity of reducing bodies is all that is desired.

240. Preservation of Sugar Juices for Analysis.—Lead sub-acetate not only clarifies the juices of canes and thus permits of

⁶³ Spencer, Handbook for Cane-sugar Manufacturers, 4th Edition, 1906: 300-306.

their more exact analytical examination, but also exercises preservative effects which enable it to be used as a preserving agent and thus greatly diminishes the amount of work necessary in the technical control of a sugar factory. Instead, therefore, of the analyst being compelled to make an examination of every sample of the juice, aliquot portions representing the different quantities can be preserved and one analysis made for all. This method has been thoroughly investigated by Edson, who also finds that the errors, which may be introduced by the use of the lead subacetate in the analytical work, may be entirely avoided by using the normal lead acetate.⁶⁴

In the use of the normal lead acetate, much less acetic acid is required in the polariscope work than when the subacetate is used. The normal lead acetate is not so good a clarifying agent as the subacetate, but its efficiency in this respect is increased by the addition of a little acetic acid. In its use, it is not necessary to remove the lead, even for the determination of the reducing bodies.

Formaldehyde has been suggested for preserving juices but care has to be exercised not to use an excess as it changes the rotation of the sugars.⁶⁵

For further details in regard to the technical determination of reducing bodies, special works may be consulted.⁶⁶

241. Direct Determination of Sugars in Canes.—The methods, which have just been described, of securing the juices of cane by pressure and of determining the sugars therein, do not give the actual percentage of sugar in the cane. An approximate result may be secured by assuming that the cane is composed of 90 parts of juice and 10 parts of cellular tissues and other insoluble matters. This assumption is approximately true in most cases, but there are often conditions arising which render the data calculated on the above assumption misleading. In any particu-

⁶⁴ *Journal of Analytical Chemistry*, 1890, 4 : 381.

⁶⁵ Hawaiian Sugar Planters' Association, Division of Agriculture and Chemistry, Experiment Station, Bulletin 23, 1908.

⁶⁶ Spencer, *Handbook for Cane-sugar Manufacturers*, 4th Edition, 1906 : 15 et seq.

lar case in order to be certain that the correct percentage of sugar is secured it will be necessary to determine the fiber in the cane. This is an analytical process of considerable labor and especially so on account of the difficulty of securing samples which represent the average composition of the cane. The fibrous structure of the canes, the hardness of their external covering and the toughness of their nodes or joints render the sampling extremely difficult. Moreover, the content of sugar varies in different parts of the cane. The parts nearest the ground are, as a rule, richer than the upper joints and this is especially true of sugar cane. In order, therefore, to get a fair sample, even of a single cane, all parts of it must be considered. Several methods of the direct determination of sugar in canes have been proposed and will be described below.

242. Methods of Determination.—Even the finely divided material obtained by shredding in the apparatus described on page 10 is not suited to give an instantaneous diffusion for polarization as is done by the finely ground beet pulp to be described further on. For the determination of sugar a proper weight of the pulp obtained as described above, taken after thorough mixing, is placed in a flask graduated properly and treated with water.⁶⁷

The flask in which the mixture takes place should be marked to compensate for the volume of the fiber of the cane. When the normal weight of cane is used for the ventzke scale, *viz.*, 26.048, the flask should be graduated at 102.6 cubic centimeters. If double the normal weight be used, the flask should be graduated at 205.2 cubic centimeters. This graduation is based on the assumption of the presence of fiber amounting to 10 per cent. of the weight of the cossettes. The fiber is so nearly the density of the juice obtained as to be regarded as one gram equal to one cubic centimeter. The flask is at first filled almost full of water and then warmed to near the boiling point for an hour with frequent shaking. It is then filled to a little above the mark, the

⁶⁷ Bulletin d l'Association des Chimistes de Sucrerie et de Distillerie, 1884, 2 : 369.

contents well mixed and warmed for ten minutes more with frequent shaking. After cooling, the volume is made up to the mark, well shaken and poured upon a filter. The filtrate is collected in a sugar flask marked at 50 and 55 cubic centimeters. When filled to the first mark a proper quantity of lead acetate is added, the volume completed to the second mark with water, the contents of the flask well shaken, poured upon a filter and the filtrate polarized in the usual way.

The reducing sugar is determined in an aliquot part of the filtrate by one of the alkaline copper methods. Spencer advises the extraction of the pulp by successive treatments with boiling water.⁶⁸ Approximately 100 grams of the pulp is extracted seven times with 200 grams of water and the residue finally pressed dry and the liquids all united polarized in a 400 millimeter tube. The percentage of sugar may be calculated or read directly from a table. Other methods of procedure may also be consulted.⁶⁹

243. Determination by Drying and Extraction.—Instead of the diffusion and polarization method just described, the fine pulp obtained may be dried, the dried residue ground in a drug mill and extracted with aqueous alcohol or with water.

To facilitate the calculation when this method is employed, the water content of a small portion of the well sampled pulp is determined. The rest of the pulp is dried, first for a few hours at a temperature not above 60° or 70°, and then at the temperature of boiling water, either in the open air or a partial vacuum, until all the water is driven off. The dried residue can then be preserved in well stoppered bottles for the determination of sugar at any convenient period. The finely ground dried residue for this purpose is placed in an extraction apparatus and thoroughly exhausted with 80 per cent. alcohol. The extract is dried and weighed, giving the total weight of all sugars present. After weighing, the extract is dissolved in water, made up to a definite volume and the reducing sugars determined in an aliquot portion thereof by the usual methods. The weight of reducing sugars

⁶⁸ Handbook for Cane Sugar Manufacturers, 4 Edition : 114.

⁶⁹ International Sugar Journal, Oct. and Nov. 1908, and Aug. 1909.

found, calculated for the whole extract, deducted from the total weight of this extract will give the weight of the sucrose in the sample. From this number the content of sugar in the original cane is determined from the percentage of water found in the original sample. For control analytical methods this process is unpractical but it is to be applied in the determination of sugars in dried materials.

Example.—In a sample of finely pulped canes the content of water is found to be 76.5 per cent. The thoroughly dried pulp is ground and extracted with aqueous alcohol. Five grams give two and five-tenths grams of the extract. The extract is dissolved in water, made up to a definite volume and the reducing sugars determined in an aliquot part and calculated for the whole, amounting to 150 milligrams. The extract is therefore composed of 2.35 grams of sucrose and 0.15 gram of reducing sugars. The calculation is now made to the original sample which contained 76.5 per cent. of water and 23.5 per cent. of dry matter, as follows:

$$5 : x :: 23.5 : 100, \text{ whence } x = 21.27,$$

the weight of the original material corresponding to five grams of the dry substance. The original composition of the sample is therefore expressed by the following numbers:

	Per cent.
Sucrose.....	11.1
Reducing sugars	0.7
Water.....	76.5
Fiber (insoluble matter)	11.7

244. Examination of the Bagasse.—The method just described for the examination of canes may be also applied to the analysis of bagasses, with the changes made necessary by the increased percentage of fiber therein. On account of the large surface exposed by the bagasse, the sampling, shredding and weighing should be accomplished as speedily as possible to avoid loss of moisture. •

The optical examination of bagasses is rendered difficult by reason of the uneven pressure to which the canes are subjected. With fairly good milling in technical work the bagasses will have

at least thirty per cent. of fiber. The method for the polariscopic examination is therefore based upon that assumption, but the volume of the solution must be changed for varying percentages of fiber in the bagasse. On account of the smaller percentage of sugar, it is convenient to take double or three times the normal weight of the bagasse for examination. Since large sugar flasks are not commonly to be had the diffusion of the bagasse may be conducted in a quarter liter flask. In a quarter liter flask place 52.096 grams of the finely shredded bagasse, very nearly fill the flask with water and extract the sugar as described for canes in the foregoing paragraphs. In the weight of bagasse used there will be, in round numbers, 15 grams of fiber. When the volume of water is completed to the mark the actual content of liquid in the flask will therefore be only 235 cubic centimeters. Fifty cubic centimeters of the filtrate are placed in a sugar flask marked at 50 and 55 cubic centimeters, the proper quantity of lead acetate solution added the volume completed to the upper mark, the contents of the flask well shaken, filtered and polarized in a 200 millimeter tube. Assume the reading obtained to be four degrees and increase this by one-tenth for the increased volume of solution about 50 cubic centimeters. The true reading is therefore four degrees and four-tenths. This reading, however, must be corrected, because the original volume instead of being 200 cubic centimeters, is 235 cubic centimeters. The actual percentage of sugar in the sample examined is obtained by the following proportion:

$$200:235 = 4.4:x.$$

The correct reading is therefore $5^{\circ}.2$, the percentage of sugar in the sample examined.

The results obtained by the method just described may vary somewhat from the true percentage by reason of the variation of the content of fiber in the bagasse. It is, however, sufficiently accurate for technical control in sugar factories and on account of its rapidity of execution is to be preferred for this purpose. More accurate results would be obtained by drying the bagasse, and proceeding with the examination in a manner entirely

analogous to that described for the extraction of sugar from dried canes by aqueous alcohol. In both instances the reducing sugar is determined in the manner already mentioned.

A modified and improved procedure in the analysis of bagasse has been devised by Spencer.⁷⁰

245. Determination of Fiber in Cane.—In estimating the content of sugar in canes by the analysis of the expressed juices, it is important to make frequent determinations of the fiber for the purpose of obtaining correct data for calculation. In periods of excessive drought, or when the canes are quite mature, the relative content of fiber is increased, while, on the other hand, in case of immature canes, or during excessive rainfalls, it is diminished. The chief difficulty in determining the content of fiber in canes is found in securing a representative sample. On account of the hard and fibrous nature of the envelope and of their nodular tissues, canes are reduced to a fine pulp with great difficulty by the apparatus in ordinary use. A fairly homogeneous pulp, however, may be obtained by means of the shredder described on page 10. The canes having been shredded as finely as possible, a weighed quantity is placed in any convenient extraction apparatus and thoroughly exhausted with hot water. The treatment with hot water should be continued until a few drops of the extract evaporated on a watch glass will leave no sensible residue. The residual fiber is dried to constant weight at the temperature of boiling water, cooled in a desiccator and rapidly weighed and the percentage of fiber calculated from the data obtained. On account of the great difficulty of securing a homogeneous pulp, even with the best shredding machines, the determination should be made in duplicate or triplicate and the mean of the results entered as the percentage of fiber. The term fiber as used in this sense, must not be confounded with the same term employed in the analysis of fodders and feeding stuffs. In the latter case the term is applied to the residue left after the successive treatment of the material with boiling, dilute acid and

⁷⁰ *Journal of Industrial and Engineering Chemistry* 1910, 2 : 255.

alkali. The analysis of canes for feeding purposes is conducted in the general manner hereinafter described for fodders.

246. Estimation of Sugar in Sugar Beets.—The methods employed for the determination of the sugar content of beets are analogous to those used for canes, with such variations in the method of extraction as are made possible and necessary by the difference in the nature of these sacchariferous plants. The sugar beet is more free of fiber and the hard and knotty substances composing the joints of plants are entirely absent from their composition. For this reason they are readily reduced to a fine pulp, from which the sugar is easily extracted. The analytical processes are also greatly simplified by the complete absence of reducing sugars from the juices of healthy beets. The only sugar aside from sucrose which is present in these juices is raffinose, and this is not found in healthy beets, except when they have been injured by frost or long keeping. In practical work, therefore, the determination of sucrose completes the analysis in so far as sugars are concerned. Two methods of procedure will illustrate all the principles of the various processes employed, *viz.*, by expressing the juice of the beet and by operating on the pulp.

247. Estimation of Sucrose in the Expressed Juice.—In the first method the beets are reduced by any appropriate machine, to a fine pulp, which is placed in a press and the juice extracted. In this liquor, after clarification with lead subacetate, the sucrose is determined by the polariscope. The methods of measuring, clarifying and polarizing are the same as those described for saccharine juices in previous paragraphs. The percentage of juice in the sugar beet varies from 88 to 95. An average factor is 93. The corrected polariscopic reading obtained multiplied by 0.93 will give the percentage of sugar in the beet. A very efficient press for securing a maximum amount of juice is shown in Fig. 63.

Example.—The solids in a sample of beet juice, as measured by a brix spindle, are 17.5 per cent. Double the normal weight of the juice is measured from a sucrose pipette, placed in a sugar flask, clarified, the volume completed to 100 cubic centimeters, the

contents of the flask well shaken and filtered. The polariscopic reading obtained is $29^{\circ}.0$. Then $(29.0 \div 2) \times 0.93 = 13.5 =$ percentage of sucrose in the beet.

248. Analysis of Beet Pulp by Cold Diffusion.⁷¹—**DIRECT METHODS.**—In the usual methods the per cent. of sugar is determined directly in the beet pulp. These methods may be classified according to the solvent used for extraction, namely, water or alcohol, and these may be again divided into extraction in the cold and with heat. So many slight modifications of these different methods are known and used that all of them can not be discussed; only statements of the representative methods can be given.

WATER METHODS.—Cold-Water Digestion Method of Pellet.—Weigh the normal amount of beet cuttings, 26 or 26.048 grams, and transfer to a large-mouth flask (Fig. 64) with a mark at 200.6 cubic centimeters.⁷² Add five cubic centimeters of basic lead acetate solution, shake and add water up to the shoulder of the flask. Mix the contents by rotating in the hand and allow to stand 25 minutes in order to expel the air bubbles. Beat down the collected froth with an ether spray and fill the flask to the mark with water. Then shake the contents vigorously, placing the hand over the mouth of the flask, filter, and polarize in a 200 or 400 millimeter tube after adding a drop of acetic acid.

For this method it is necessary to have the finest divided pulp possible and it is also advisable to have a pear-shaped flask (Fig. 64) instead of the ordinary round, ball-shaped one in general use, so that the entrained air bubbles may seek the surface and not remain on the sides of the vessel. This method has been much criticized because a finely divided pulp is necessary to prevent an imperfect extraction of the sugar, and also because ordinary shaking will not disengage all of the air bubbles from the pulp, thereby causing the use of a smaller quantity of water to fill the flask to the mark, and thus giving too high results for sugar in the beet.

⁷¹ Bryan, Bureau of Chemistry, Bulletin 146, 1911 : 17-22.

Neue Zeitschrift für die Rübenzucker-Industrie 1879, 2 : 1, 17, 287
692 and 3 : 242.

Zeitschrift des Vereins der Rübenzucker-Industrie 1909, 59 : 627.

Bulletin d'Association des Chimistes de Sucrerie et de Distillerie
1894, 12 : 380.

⁷² Numerous experiments have shown that the marc of the normal weight of average beets occupies about 0.6 cc of space.

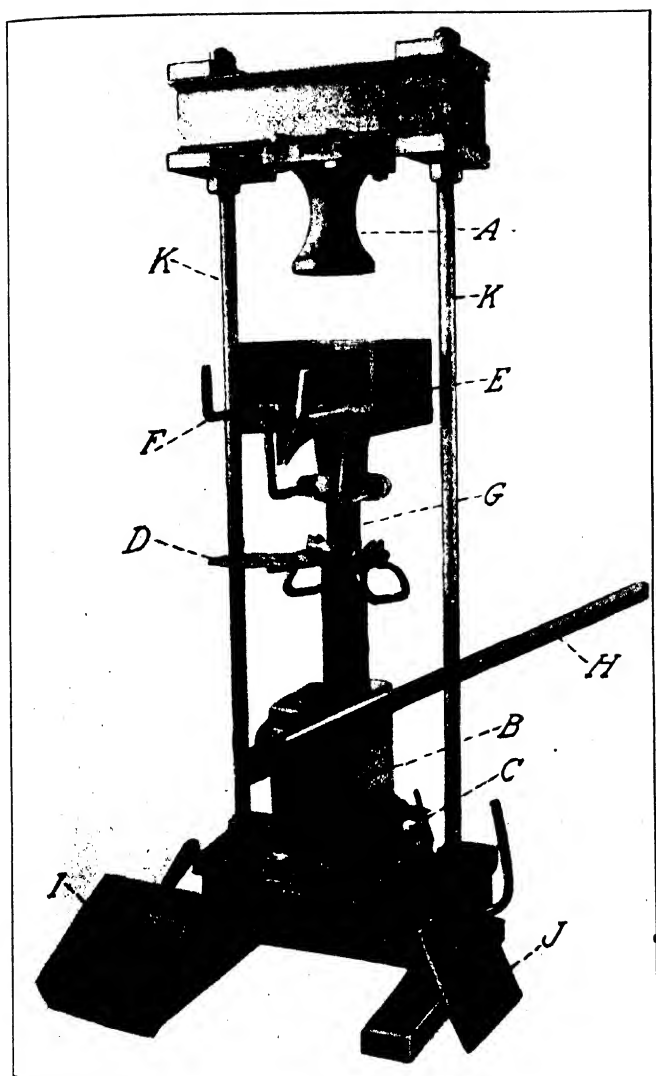


Fig. 63.—Hydraulic Press for Beet Analysis.

A, Head block. *B*, Hydraulic jack with ram *G*. *C*, Release valve of jack. *D*, Stand for vessel to receive juice. *E*, Receiving box fastened to ram *G*. *H*, Handle for pumping up jack. *I*, *J*, Porous box and cover for beet sample.

Cold-Water Digestion Method of Sachs Le Docte.—This is a modification of Pellet's method for the purpose of removing the error resulting from the entrained air. Place 26 grams of the

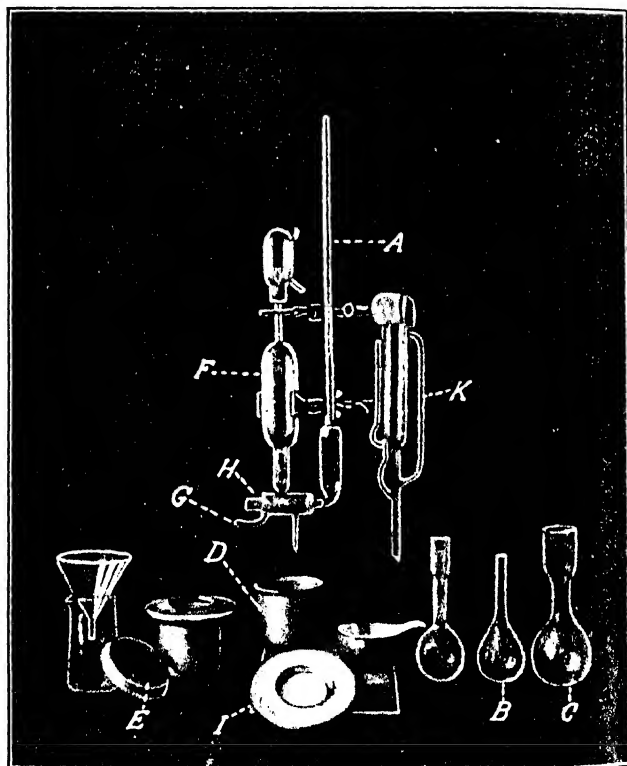


Fig. 64.—Apparatus for Beet Analysis.

A, Sucrose pipette. *B*, Sugar flask. *C*, Flask for cold or hot water digestion. *D*, *E*, *I*, Metal cups and covers for the Sachs Le Docte method. *F*, Automatic pipette for the Sachs Le Docte method. *K*, Modified Soxhlet extractor for alcohol extraction method.

fine beet pulp into a tin-lined copper vessel (*D*, Fig. 64), add 5 cubic centimeters of basic lead-acetate solution and 172 cubic centimeters of water. Put the cover *E* in place and shake the

whole vigorously, then allow it to stand for three minutes, remove the cover, filter, and polarize the solution in a 200 millimeter tube after adding a drop of acetic acid and double the reading, or polarize in a 400 millimeter tube, in which case the reading gives the per cent. of sugar in the beet.

In this method it is assumed that beets contain 95 per cent. of juice with an average specific gravity of 1.07. The volume of the juice contained in a normal weight would then be

$$\frac{26 \times 0.95}{1.07} = 23.08 \text{ cc.}$$

and (200—23.08 cubic centimeters) 176.92 cubic centimeters of water would be necessary to complete the volume to 200 cubic centimeters. A special pipette (*F*, Fig. 64) has been designed to deliver this amount. A quarter turn of the stopcock opens the entrance *G* for the lead acetate, so that five cubic centimeters can be measured, then another quarter turn opens the water entrance *H* to fill the burette to the overflow, and a half turn delivers the whole into the dish.

As in the preceding method, the beet pulp must be very fine, otherwise an imperfect extraction will occur. This method is also open to criticism in that all beets do not contain 95 per cent. of juice and all beet juice does not possess a specific gravity of 1.07. It is rightly claimed, however, that the errors introduced by the use of these constants are so small that they will fall within the limits of accuracy of the readings.

Hot-Water Digestion Method.—Weigh 52 grams of the beet cuttings and transfer them with water to a large-mouth flask (*C*, Fig. 64) of 201.2 cubic centimeters content; add from five to 10 cubic centimeters of lead subacetate solution, fill the flask to the mark with hot water, and shake. Immerse the flask in a water bath at 80° C. and shake at intervals. Add water from time to time so that at the end of the heating, about 30 minutes, the water in the flask is a little above the mark. Remove the flask from the water bath and allow it to cool to standard temperature; add a sufficient quantity of concentrated acetic acid to make the solution very slightly acid (generally less than 0.5 cubic centimeter is necessary) and a few drops of ether to break the foam; complete the volume. Mix thoroughly, filter, and polarize in a 200 millimeters tube.

With this method a coarser beet pulp can be used than for the cold-water methods, but over 30 minutes may be necessary for digestion, if very coarse cuttings are used. To obtain correct results, care must be exercised to make up to volume at the

standard temperature of 20° C. (if the instrument and flasks are standardized at that temperature) and to digest the beet cuttings with as large a quantity of water as possible. Not over five cubic centimeters in any case should be added during digestion and final cooling to complete the volume. Where smaller quantities of water are used during digestion and then a large quantity of water is added at the last to make to volume, the sugar has not become equally diffused and the results are too low. Differences of over 1 per cent. in sugar content are often caused by lack of care in this particular.

Hot-Water Digestion Method of Sachs Le Docte.—The procedure in the Sachs Le Docte cold-water extraction method is modified as follows for hot digestion: The weighing and the vessels used are the same, also the quantities of lead subacetate and water are the same, namely, 177 cubic centimeters. A special rubber disk cover (1, Fig. 64) is provided for the digestion vessels. Put this in place and after shaking the vessel immerse it in a water bath kept at 80° C. for 30 minutes, or for 25 minutes if the temperature is 85° C. The temperature during extraction should not, however, exceed this figure. Remove the cups and immerse in cold water, bringing the temperature down to 20° C., shake, remove the covers, filter, and polarize, after adding a drop or so of acetic acid.

The chance of error due to contained air or unequal diffusion of the pulp is removed by this method. As in the former case the cuttings need not be so fine as with the cold-water extraction methods.

Herzfeld's Modification of the Sachs Le Docte Method.—Instead of the tin-coated copper beakers used in the preceding method, Herzfeld uses an extraction vessel of nickel-plated sheet iron made as shown in Fig. 65. The vessel is round. He also uses small weighing glasses, holding 26 grams of material, which can be introduced with the beet cuttings into the extraction vessel. These watch glasses are filled to equal weight and numbered consecutively, as are also the extraction vessels. The procedure is as follows:

Weigh 26 grams of the beet pulp on a watch glass and transfer to the extraction vessel, then run in 177 cubic centimeters of dilute basic lead-acetate solution (5 parts of basic lead-acetate solution, brix 53.5, to 100 parts of water), shake and place a stopper which has been covered with tinfoil lightly in the opening. Submerge the whole in a water bath at 75° to 80° C. for 30 minutes, shaking intermittently. When all air has been expelled (gen-

erally after five minutes), tighten the stopper in the vessel. At the expiration of the time remove and cool. Take out the stopper after shaking thoroughly, filter, and polarize in a 400 millimeter tube, after addition of a drop of acetic acid, to determine the per cent. of sugar in the beet.

This method does not require very fine pulp and is open to few chances of error. It, together with the Sachs Le Docte method, has a decided advantage over the other hot digestion method, in that there is little chance of a loss of sample by the container breaking. When working with glass flasks one is likely to ruin many determinations. For quick work and when many samples are to be run, large heating and cooling vessels can be used and the dishes taken from the one and placed immediately in the other

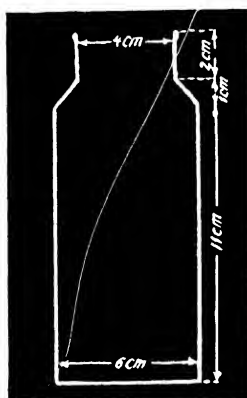


Fig. 65.—Herzfeld's Metallic Beaker.

to cool, without risk of losing the determination, thereby saving considerable time. This method has been adopted by the Society of the German Sugar Industry, and with a few modifications in apparatus, but not of procedure, by a number of other sugar associations of Europe.

Alcohol Methods.—The methods using alcohol as the solvent may be divided into two classes—digestion and extraction methods. The former is again divided into hot and cold digestion methods.

Cold Alcohol Digestion Method.—Weigh 52 grams of the beet cuttings and transfer to a flask (having a capacity of 201.2 cubic

centimeters) with 90 per cent. alcohol, add 4 cubic centimeters of lead subacetate solution and shake, then add more 90 per cent. alcohol with shaking to remove the air bubbles, complete the volume, allow to stand half an hour, and add alcohol if a decrease in volume is noted. Thoroughly shake the flask and filter, keeping the filter covered to prevent loss by evaporation. Polarize in a 200 millimeters tube.

With this method very fine pulp is necessary and the greatest care must be exercised to remove all of the air from the beet cuttings to insure an even digestion. For accurate readings the polarization should be made at the standard temperature of the instrument and flasks and allow of no evaporation. This method is but little used.

Hot Alcohol Digestion Method.—The same procedure is followed as in the preceding method up to the point of adding more alcohol. In this method add only enough 90 per cent. alcohol to fill the flask three-fourths full. Then connect the flask with a return condenser, place in a water bath, and allow to boil for 20 minutes. Cool the flask and contents to the standard temperature and bring up to the mark with 90 per cent. alcohol, shake and allow to stand for awhile, filter, and polarize.

This method does not require so fine a pulp as the former one and is not open to error from entrained air bubbles, but to obtain correct results it must be worked carefully to prevent evaporation and changes of temperature during polarization.

Alcohol Extraction Method.—The alcohol extraction method has been recognized as the standard method for sugar determination and is the one with which other methods are compared, but its execution is difficult and the results are liable to error if the greatest care is not exercised. For the inexperienced chemist it is not a suitable method, but by familiarizing oneself with its details and difficulties, correct results can be obtained.

A Soxhlet extraction apparatus is necessary for this method. The usual form has been improved by providing the siphon tube with a short tube and cork as shown in Fig. 64, K. In this way one can test the progress of the extraction by withdrawing a sample without interfering with the work. The best procedure for this method is to weigh 26 grams of the beet pulp and transfer to a 100 cubic centimeters flask with about 50 cubic centimeters of 90 per cent. alcohol and from 3 to 5 cubic centimeters of basic lead-acetate solution. Connect a return condenser to the flask and place in a boiling water bath for from 10 to 15 minutes. Then pour the whole into the extractor, washing out the flask with

fresh portions of 90 per cent. alcohol. A return condenser is connected with the Soxhlet extractor and also a 100 cubic centimeter flask, the latter by means of a cork. Add more 90 per cent. alcohol until the siphon is started and the lower flask is about three-fourths full. Place the containing flask in a covered water bath held at a heat that will make the alcohol boil freely. Continue the extraction for from one to four hours, or until a test of the alcohol in the extractor gives no color with *a*-naphthol solution. Remove the flask and add 90 per cent. alcohol to the mark after cooling to the standard temperature, shake and filter. Polarize in a 200 millimeter tube.

Care must be exercised to prevent evaporation and changes of temperature and also to use only a minimum amount of basic lead acetate, generally nearer 3 cubic centimeters than 5 cubic centimeters, for clarification. By digesting the beet pulp with the alcohol before extraction, the time of extraction is greatly shortened, the pulp becomes thoroughly impregnated with the alcohol, and all air is removed, resulting in a good extraction of the whole material. If the pulp is fine and tends to clog the siphon, alcohol-washed cotton may be used as a plug in the extractor before adding the beet pulp, and a fine mesh screen may be placed over the pulp to keep the whole compact in the extractor.

To determine whether all of the sugar has been extracted or not the following qualitative test is used:

a-naphthol test for sugars.—Add four or five drops of a 20 per cent. alcoholic *a*-naphthol solution to a few drops of the alcohol coming from the extractor and 2 cubic centimeters of water contained in a small test tube. Shake well, tip the test tube, and allow from 2 to 5 cubic centimeters of colorless concentrated sulfuric acid to flow down the side of tube; then hold the tube upright, and if sucrose is present a color varying from a faint to a deep violet will be noted at the junction of the two liquids. On shaking, the whole solution becomes a blue violet color. This test is suitable for the results required of it in this work, but it must be remembered that other sugars and substances besides sucrose give this color reaction.

Discussion of Methods.—For a number of years the proper methods for determining sugar in the beet have been discussed by sugar chemists, especially as to the relative merits of alcohol and of water extraction. As a general rule German sugar chemists favored alcohol methods, while the French favored water methods. Within the last year a truce has been declared

and it is acknowledged that for very accurate or control work the alcohol extraction method should be used, but for general work, one of the hot-water digestion methods will give good results and the analyst is less likely to introduce errors in the manipulation. With the average sample of beets, the two methods when carefully applied will yield duplicate results but in the case of very abnormal beets one method might give higher figures than another. The instantaneous methods (cold extraction) are only suitable with very fine pulp and the results can be depended upon only when they have been checked against a standard method. With these instantaneous methods as many samples of beets can be analyzed per day as by the indirect method, and it is claimed by many that the Sachs Le Docte hot digestion method or the Herzfeld modification will yield as many determinations in a given time as the indirect method, with the same amount of work, provided the laboratory is fitted for the work.

249. Instantaneous Diffusion.—In the second process employed

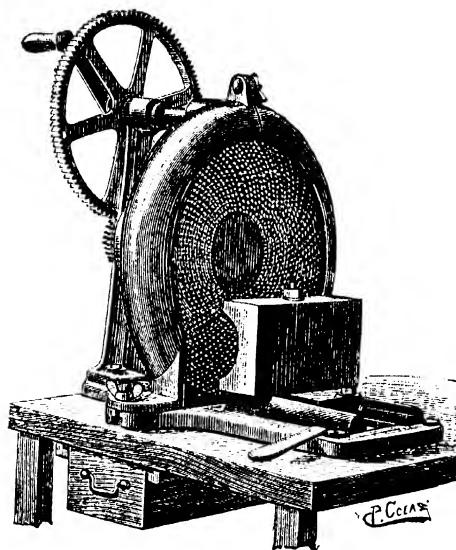


Fig. 66. Apparatus for Pulping Beets.

for determining the sugar content of beets, the principle involved depends on the use of a pulp so finely divided as to permit of the

almost instant diffusion of the sugar present throughout the added liquid. This diffusion takes place even in the cold and the process thus presents a convenient and rapid method for the accurate determination of the percentage of sugar in beets. The pulping is accomplished by means of the machine described on page 10, or the one shown in Fig. 66. Many other forms of apparatus have been proposed and described for securing a uniform pulp but they do not involve any new principle. The beet is pressed against the rapidly revolving rasp by means of the grooved movable block and the finely divided pulp is received in the box below. These machines afford a pulp which is impalpable and which readily permit an almost instantaneous diffusion of its sugar content.

250. Determination of Sugar in Mother Beets.—In selecting mother beets for seed production, it is necessary to secure only

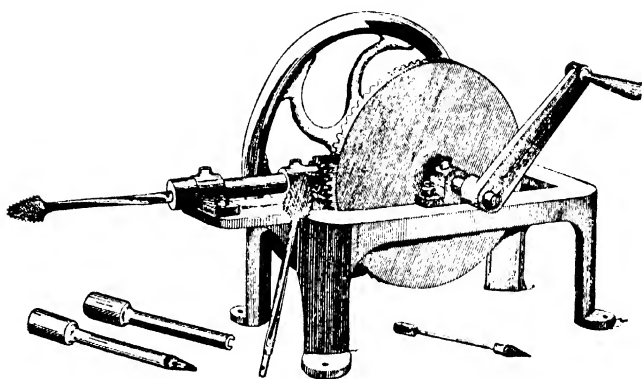


Fig. 67.—Rasp for Sampling Mother Beets.

those of a high sugar content. This is accomplished by boring a hole about two and a half centimeters in diameter obliquely through the beet by means of the apparatus shown in Fig. 67.

The beet is not injured for seed production by this process,

and the pulp obtained is used for the determination of sugar. The juice is expressed by means of the small hand press shown in Fig. 68. Since only a small quantity of juice is obtained, it is advisable to prepare it for polarization in a sugar flask marked at 50 cubic centimeters. The density of the juice, by reason of its small volume, is easiest obtained by the hydrostatic balance, as described in paragraph 67. In lieu of this, the juice may be quickly weighed in a counterbalanced dish on a balance giving results accurate to within one milligram. The rest of the analytical process is similar to that already described.

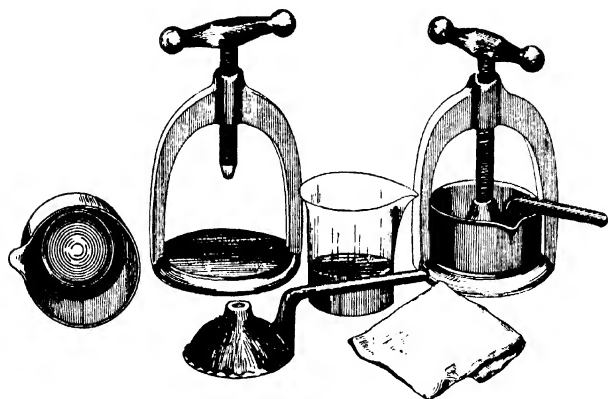


Fig. 68.—Hand Press for Beet Analysis.

251. Aqueous Diffusion.—The process of instantaneous aqueous diffusion may also be applied to the examination of mother beets. For this purpose the beets are perforated by a rasp, devised by Keil, shown lying on the floor in Fig. 67, the characteristics of which are shown in Fig. 69. The conical end of the rasp is roughened in such a way as to reduce the beet to an impalpable pulp. This end is fastened by a bayonet fastening to the cylindrical carrier or arm in such a way that, by means of a groove in the conical end of the rasp, the pulp is introduced into the cylinder. The cylinder is provided with a small piston by

means of which the pulp can be withdrawn when the cylindrical portion of the rasp is detached from the driving machinery. It is important that the rasp be driven at a high rate of speed, *viz.*, from 1,500 to 2,000 revolutions a minute. The sample of pulp at this rate of revolution is taken almost instantly, and with skilled manipulators the whole operation of taking a sample, removing the rasp by means of its bayonet fastenings, withdrawing the sample of pulp and replacing the rasp ready for another operation does not consume more than from ten to twenty seconds. From three to four samples may thus be taken in a minute. The samples of pulp as taken are dropped into num-

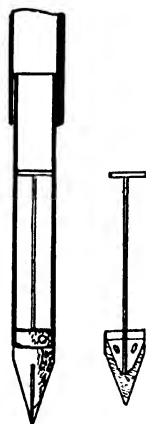


Fig. 69

bered dishes corresponding to the numbers on the beets. One-quarter of the normal weight for the polariscope is used for the analysis. The pulp is placed in a 50 cubic centimeter flask, water and lead subacetate added, the flask well shaken, filled to the mark with water, again well shaken, the contents thrown on the filter, and the filtrate polarized in a 400 millimeter tube, giving the direct percentage of sugar. For practical purposes the percentage of marc in the beet may be neglected. If the polarization take place in a 200 millimeter tube the number obtained should be multiplied by two for the content of sugar.

In numbering sugar beets which are to be analyzed for seed production, it is found that a small perforated tin tag bearing a number may be safely affixed to the beet by means of a tack. It is not safe to use paper tags as they may become illegible by becoming wet before the sorting of the beets is completed. Where from 1,000 to 2,000 beets are to be examined in a day, the number of the beets and the dishes corresponding thereto must be carefully controlled to avoid confusion and mistakes.

252. Analysis of Sirups and Massecuites.—The general principles which control the analysis of sirups and massecuites are the same whether these products be derived from canes or beets. In the case of the products of canes, the sirups or massecuites contain chiefly sucrose, invert sugar, and other copper reducing bodies, inorganic matters and water. In the case of products derived from sugar beets the contents are chiefly sucrose, inorganic matters, a trace of invert sugar, raffinose and water. The principles of the determination of these various constituents have already been described.

253. Specific Gravity.—The specific gravity of sirups and molasses can be determined by the spindle in the usual way, but in the case of molasses which is quite dense, the spindle method is not reliable. It is better, therefore, both in molasses and massecuites, to determine the density by dilution. For this purpose, as described by Spencer, a definite weight of material, from 200 to 250 grams, is dissolved in water and the volume of the solution completed to half a liter. A portion of the solution is then placed in a cylinder and the quantity of total solids contained therein determined in the usual way by a brix or specific gravity hydrometer. In case 250 grams of the material be used the calculation of the brix degree for the original material is conducted according to the following formula:

$$x = \frac{G \times B \times V}{W}$$

In the above formula x is the required brix degree, V the volume of the solution, B the observed brix degree of the solution.

and G the corresponding specific gravity obtained from the table on page 94.

254. Determination of Water.—The accurate determination of water in sirups and massecuites is a matter of considerable difficulty. The principles of conducting the process (26), applicable also to the determination of water in honeys and other viscous liquids, are as follows: In all cases where invert sugar is present the drying should be conducted at a temperature not exceeding 75° or 80°. In dense molasses and massecuites a weighed quantity should be dissolved and made up to a definite volume and an aliquot portion taken for the determination. In order to secure complete desiccation at a low temperature, the drying should be accomplished in partial vacuum (pages 22, 23). The process of desiccation should be conducted in shallow, flat-bottom dishes which may be conveniently and cheaply made of aluminum and the process is hastened by filling the dish previously with thoroughly dried fragments of pumice stone. When the samples do not contain any invert sugar the desiccation can be safely accomplished at the temperature of boiling water. Drying should be continued in all cases until practically constant weight is obtained.

255. Determination of Ash.—Ash is an important constituent of the sirups, molasses, and massecuites from canes and exists in very much larger quantities in the same products from beets. The ash may be determined directly by careful incineration, but it is customary to add a few drops of sulfuric acid, sufficient to combine with all the bases present and be in slight excess. The presence of sulfuric acid is of some advantage in the beginning of the carbonization and renders the process somewhat easier of accomplishment. When sulfuric acid is used, the weight of ash obtained must be diminished by one-tenth to allow for the increased weight obtained by the conversion of the carbonates into sulfates. In general, the principles and methods described on pages 46 et seq. are to be employed. The use of a little olive oil on top will stop frothing and danger of loss.

256. Determination of Reducing Sugars in Sirups, Molasses, and Massecuites.—The quantity of reducing sugars in the products derived from the sugar beet, as a rule, is insignificant. In the products from sugar cane there are large quantities of reducing matters which, in general, are determined by any of the standard methods already given. It has been shown by the author⁷³ that the juices of healthy sugar canes contain a small quantity of invert sugar, but this statement has been contradicted by Beaufret.⁷⁴ The reducing bodies derived from the products of manufacture of sugar cane and sorghum deport themselves in a manner somewhat different from pure invert sugar. In the absence of definite information in respect of the constitution of these bodies, the methods applicable to dextrose and invert sugar may be applied.⁷⁵ Some interesting observations on this subject have been made by Browne and Blotin.

The copper carbonate solution, as has already been said, is peculiarly suited to the determination of reducing sugars in the presence of sucrose and the modified forms of this solution, and the methods of employing them with invert sugar, dextrose, levulose, and maltose, have already been described.

SUCROSE, DEXTROSE, INVERT SUGAR, LEVULOSE, MALTOSE, RAFFINOSE, DEXTRIN AND LACTOSE IN MIXTURES.

257. Occurrence.—Sucrose and invert sugar are found together in many commercial products, especially in raw sugars and molasses made from sugar cane, and in these products sucrose is usually predominant. They also form the principal saccharine contents of honey, the invert sugar, in this case, being the chief ingredient.

In commercial so-called grape sugar, made from starch, dextrose is the important constituent, while in the hydrolysis of starch

⁷³ Chemical Society of Washington, Bulletin 4, 1888-89 : 22 et. seq.

⁷⁴ Bulletin d l'Association des Chimistes de Sucrierie et de Distillerie, 1895, 13 : 133.

⁷⁵ Agricultural Experiment Station, Louisiana State University, Bulletin 91, 1907 : 17.

by a diastatic ferment, maltose is principally produced. In the manufacture of commercial glucose by the saccharification of starch with hydrochloric acid, the dextrin and dextrose constitute the dominant products, while in the similar substance midzu ame, maltose and dextrose are chiefly found, and only a small quantity of dextrose.⁷⁶ In honeys derived from the exudations of coniferous trees are found also polarizing bodies not enumerated above and possibly of a pentose or dextrinoid character. The investigations of Browne, however, show that no notable quantities of pentosans are found in honeys.⁷⁷ In evaporated milks are often found large quantities of added sucrose in addition to the natural sugar therein contained. These mixtures of carbohydrates often present problems of great difficulty to the analyst, and the following paragraphs will be devoted to an elucidation of the best approved methods of solving them.

OPTICAL METHODS.

258. Sucrose and Invert Sugar.—The chemical methods of procedure to be followed in the case of a sample containing both sucrose and invert sugar have been given in sufficient detail. When, however, it is desirable to study further the composition of the mixture, important changes in the method are rendered imperative. While the estimation of the sucrose and the total invert sugar, or the sum of the dextrose and levulose, is easy of accomplishment the separate determination of the dextrose and levulose is not so readily secured. In the latter case the total quantity of the two sugars may be determined, and after the destruction or removal of one of them the other be estimated in the usual way; or in the mixture the levulose can be determined by the variation in its gyrodyn, caused by changes of temperature.

259. Optical Neutrality of Invert Sugar.—The gyrodyn of levulose decreases as the temperature rises, and at or near a temperature of $87^{\circ}.2$, it becomes equal to that of dextrose, and, there-

⁷⁶ *Journal of the American Chemical Society*, 1880, **2** : 387; *Agricultural Science*, 1892, **6** : 57.

⁷⁷ *American Chemical Journal*, 1891, **13** : 24.

Bureau of Chemistry, Bulletin 110, 1908 : 50.

fore, pure invert sugar composed of equal molecules of levulose and dextrose is optically neutral to polarized light at that temperature. On this fact Chandler and Ricketts have based a

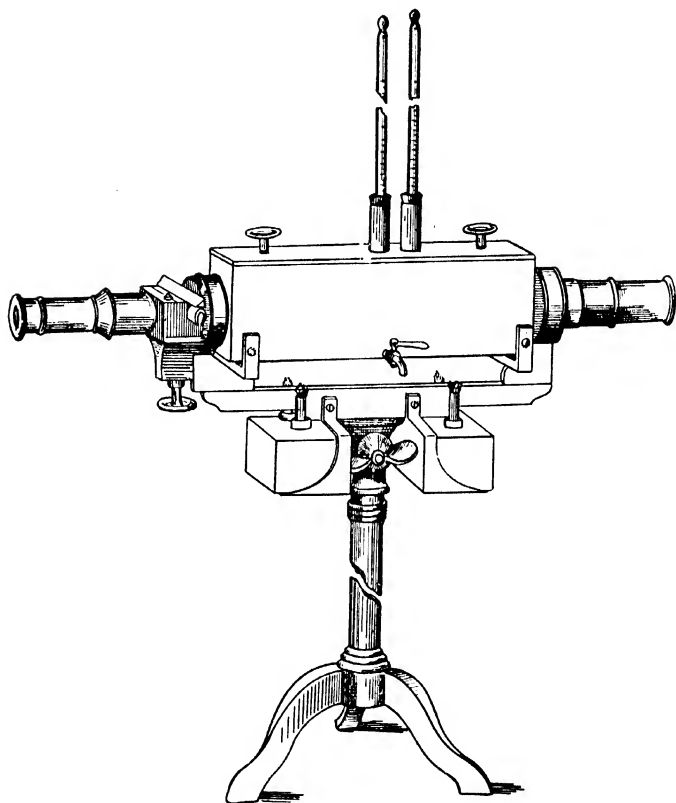


Fig. 70.—Chandler and Ricketts' Polariscopes.

method of analysis which excludes any interference in polarization due to invert sugar.⁷⁸ To secure the polarization at approximately a temperature of 87°, a water-bath is placed be-

⁷⁸ Tucker, *Manual of Sugar Analysis*, 1883 : 287 ; Wiechmann, *Sugar Analysis*, 1893 : 51.

tween the nicols of an ordinary polariscope in such a way as to hold a tubulated observation tube in the optical axis of the instrument. The ends of the bath, in the prolongation of this axis, are provided with clear glass disks. The space between the cover glasses of the observation tube and the glass disks of the bath is occupied by the water of the bath. When this is kept at a constant temperature it does not interfere with the reading. The observation tube may be of glass, but preferably is constructed of metal plated with platinum on the inside. For the most exact work the length of the observation tube, at 87° , is determined by measurement or calculation. The bath is heated with alcohol lamps or other convenient means. The arrangement of the apparatus is shown in Fig. 70.

In a mixture of sucrose and invert sugar any rotation of the plane of polarized light at 87° is due to the sucrose alone. In a mixture of dextrose and sucrose the polarization is determined, and, after inversion, again determined at 87° . The latter number is due to dextrose alone, and the difference between the two gives the rotation due to sucrose.

260. Optical Determination of Levulose.—The determination of levulose by optical methods alone is made possible by reason of the fact that the gyrodynic of the sugars with which it is associated are not greatly affected by changes of temperature. The principle of the process, as developed by the author, rests on the ascertainment of the change in the gyrodynic of levulose when its rotation is observed at widely separated temperatures.⁷⁹ The observation tube employed for reading at low temperatures is provided with desiccating end tubes, which prevent the deposition of moisture on the cover glasses. The relations of this device to the optical parts of the apparatus are illustrated in Fig. 71.

The protecting tubes are made of hard rubber and the desiccation is secured by surrounding the space between the rubber and the perforated metal axis with fragments of potash or calcium chloride.

⁷⁹ Wiley, *Journal of the American Chemical Society*, 1896, **18** : 81; Allen, *Commercial Organic Analysis*, 4th Edition, 1909, **1** : 302.

The details of the construction are shown in a horizontal section through the center of the observation tube in Fig. 72. In this figure the observation tube, made of glass or metal, is

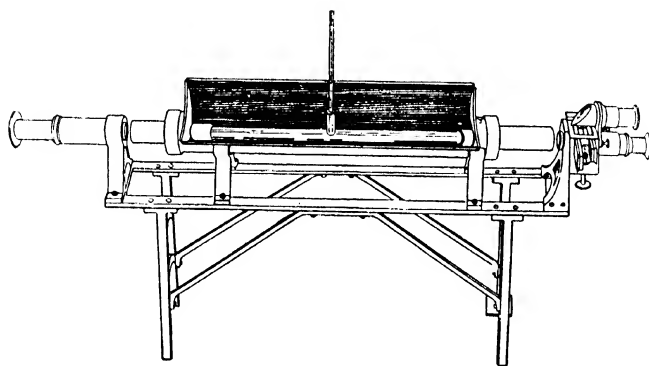


Fig. 71.—Apparatus for Polarimetric Observations at Low Temperatures.

represented by *i*, the metal jacket, by *k*. The observation tube is closed by the heavy disk *b* made of non-polarizing glass. This disk is pressed against the end of the observation tube by the

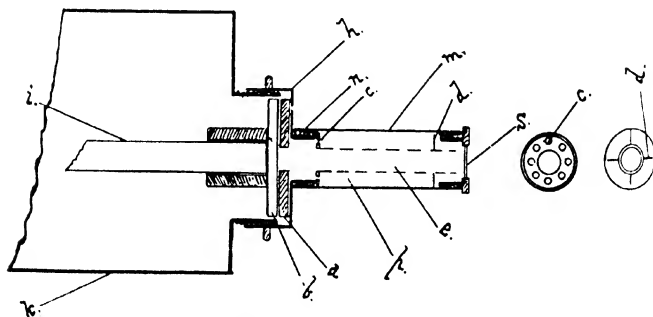


Fig. 72.—Construction of Desiccating Tube.

rubber washer *a*, when the drying system about to be described is screwed on to *k*. The apparatus for keeping the cover glass

dry is contained in the hard rubber tube *m* and consists of a perforated cylinder of brass *e*, supported at one end by the perforated disk *c'* and at the outer ends by the arms *d*. It is closed by a cover glass of non-polarizing glass *s* and can be screwed on to the system *h* at *n*. The space *p* is filled with coarse fragments of caustic soda, potash, or calcium chlorid by removing the cover glass *s*. The perforated disk *c'* prevents any of the fragments from entering the axis of observation. When the cover glass *s* is replaced, it just touches the free end of the perforated metal tube preventing any of the fragments of the drying material from falling into the center at the outer end. When this drying tube is placed in position, the contents of the observation tube *i* can be kept at the temperature of zero for an indefinite time without the deposition of a particle of moisture either upon the glass *b* or *s*.

For determining the rotation at a high temperature the apparatus of Chandler and Ricketts (259) may be used or the following device: The polarizing apparatus shown above, Fig. 71, may be used after the V shape box is removed from the stand which is so constructed as to receive a large box covered with asbestos felt an inch thick. The observation tube is held within this box in the same way as in the one just described so that the hot water extends not only the entire length of the tube but also covers the cover glasses. In both cases the cover glasses are made of heavier glass and are much larger in diameter than found in the ordinary tubes for polariscopes. The protecting cylinders of hard rubber are not needed at high temperatures but can be left on without detriment.

The illustration, Fig. 73, shows the arrangement of the apparatus with a silver tube in position, which can be filled and emptied without removing it.

In practice the water is heated with a jet of steam and an even temperature is secured by a mechanical stirrer kept slowly in motion. With such a box it is easy to maintain a temperature for several hours which will not vary more than half a degree. The temperature for reading the hot solutions was fixed at 88°, this

being nearly the temperature at which a mixture of equal molecules of levulose and dextrose is optically inactive. In every case the sugar solutions were made up to the standard volume at the temperatures at which they were to be read and thus the variations due to expansion or contraction were avoided. When solutions are read at a high temperature, they must be made with freshly boiled water so as to avoid the evolution of air bubbles, which may otherwise obscure the field of vision.

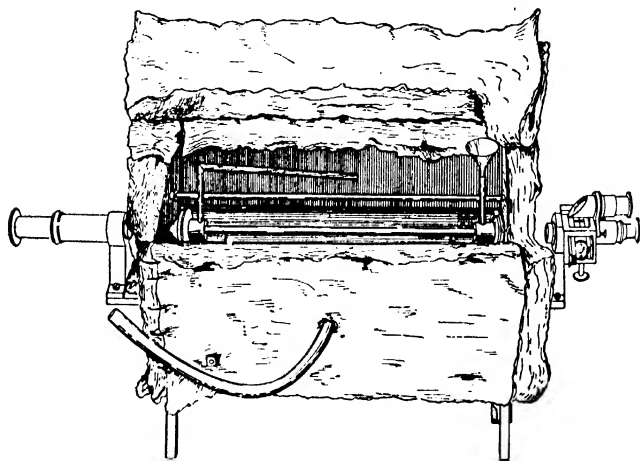


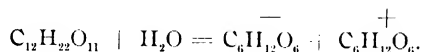
Fig. 73.—Apparatus for Polarizing at High Temperatures.

By means of the apparatus described it is easy for the analyst to make a polarimetric reading at any temperature desired. In all cases the observation tube should be left at least a half an hour and sometimes longer in contact with the temperature control media before the reading is made.

The appearance of the field of vision is usually a pretty fair index of the point of time at which a constant temperature is established throughout all parts of the system. Any variation in temperature produces a distortion of the field of vision while a constant fixed temperature will disclose the field of vision in its true shape and distinctness of outline.

Principles of the Calculation.—If 26.048 grams of pure sucrose be dissolved in water and the volume made to 100 cubic centimeters, it will produce an angular rotation of $34^{\circ}.68$ when examined in a 200 millimeter tube with polarized sodium monochromatic light. Upon the cane sugar scale of an accurately graduated shadow instrument the reading will be 100 divisions corresponding to 100 per cent. of pure sucrose.

In the complete inversion of the cane sugar the reaction which takes place is represented by the following formula:



The minus and plus signs indicate that the resulting invert sugar is a mixture of equal parts of levulose (*d* fructose) and dextrose (*d* glucose). We are not concerned here with the fact that a complete inversion of cane sugar is a matter of great difficulty nor with the danger which is always experienced of destroying a part of one of the products of inversion. They are matters which may cause a variation in the analytical data afterward, but do not affect the principles on which the process is based.

In the inversion of 26.048 grams of cane sugar there are therefore produced 13.71 grams of levulose and 13.71 grams of dextrose or, in all, 27.42 grams of the mixed sugars.

The angular rotation which would be produced by 13.71 grams of dextrose in a volume to 100 cubic centimeters and through a column 200 millimeters in length is, with sodium light, $14^{\circ}.53'$ equivalent to 41.89 divisions of the cane sugar scale. The specific rotatory power of a dextrose solution of the density given is almost exactly 53, and this number is used in the calculations.

In a mixture of the two sugars under the conditions mentioned and at a temperature of 0° the angular rotation observed is $-15^{\circ}.0.15$ equivalent to 43.37 divisions of the cane sugar scale.

The $+$ rotation due to the dextrose is $14^{\circ}.53$. Therefore the total negative rotation due to levulose at 0° is $15^{\circ}.15 + 14^{\circ}.53 = 29^{\circ}.68$. Hence the gyrodyn of levulose at 0° and in the degree of concentration noted is readily calculated from the formula

$$[\alpha]_D^{20} = \frac{29.68 \times 100}{2 \times 13.71} = 108.24.$$

Since at 88° (*circa*) the mixture of levulose and dextrose is neutral to polarized light, it follows that at that temperature the specific rotatory power of levulose is equal to that of dextrose, *viz.*, 53° .

$$[\alpha]_D^{88} = -53^\circ.$$

The total variation in the specific rotatory power of levulose, between zero and 88° , is $55^\circ.24$. The variation for each degree of temperature, therefore, of the specific rotatory power of levulose is equal to 55.24 divided by 88 , which is equal to 0.628 . From these data it is easy to calculate the specific rotatory power of levulose for any given temperature. For instance, let it be required to determine the gyrodyne of levulose at a temperature of 20° . It will be found equal to $108.24 - (0.628 \times 20) = 95.68$. The calculated rotatory power is then $[\alpha]_D^{20} = -95^\circ.68$.

In these calculations the influence of the presence of hydrochloric acid upon the rotatory power of the levulose is neglected.

Since the variation in angular rotation in the mixture at different temperatures is due almost wholly to the change in this property of the levulose it follows that the variation for each degree of temperature and each per cent. of levulose can be calculated. Careful experiments have shown that the variation in the rotatory power of levulose between 0° and 88° is represented by a straight line. For 13.71 grams per 100 cubic centimeters the variation for each degree of temperature is equal to $43.37 \div 88 = 0.49$ divisions on the cane sugar scale, or $15.15 \div 88 = 0^\circ.1722$ angular measure. If 13.71 grams of levulose in 100 cubic centimeters produce the deviations mentioned for each degree of temperature, one gram would give the deviation obtained by the following calculations:

For the cane sugar scale $0.49 \div 13.71 = 0^\circ.0357$ and for angular rotation $0.1722 \div 13.71 = 0.01256$.

The above data afford a simple formula for calculating the percentage of levulose present from the variation observed in

polarizing a solution containing levulose, provided that the quantity of levulose present is approximately fourteen grams per 100 cubic centimeters.

Example.—Suppose in a given case the difference of reading between a solution containing an unknown quantity of levulose at 0° and 88° is equal to thirty divisions of the cane sugar scale. What weight of levulose is present? We have already seen that one gram in 100 cubic centimeters produces a variation of 0.0357 division for 1° . For 88° this would amount to 3.1416 divisions. The total weight of levulose present is therefore $30 \div 3.1416 = 9.549$ grams. In the case given 26.048 grams of honey were taken for the examination. The percentage of levulose was therefore $9.549 \times 100 \div 26.048 = 36.66$ per cent.

If it be inconvenient to determine the polarimetric observations at temperatures so widely separated as 0° and 88° the interval may be made less. In the above case if the readings had been made at 20° and 70° the total variation would have been only 50/88 of the one given, *viz.*, 17.05 divisions of the cane sugar scale. The calculation would then have proceeded as follows:

$$0.0357 \times 50 = 1.785.$$

Then, $17.05 \div 1.785 = 9.552$ grams of levulose, from which the actual percentage of levulose can be calculated as above.

With honeys the operation is to be conducted as follows:

Since honeys contain approximately twenty per cent. of water and in the dry substance have approximately 45 per cent. of levulose, about 38.50 grams of the honey should be taken to get approximately 13.8 grams of levulose.

In the actual determination the calculations may be based on the factors above noted, but without respect to the degree of concentration. If half the quantity of dextrose noted be present its specific rotatory power is only reduced to about $52^\circ.75$, and this will make but little difference in the results. In the case of honey 13.024 grams of the sample are conveniently used in the examination, half the normal weight for the ventzke sugar scale. The error, however, due to difference in concentration is quite

compensated for by the ease of clarification and manipulation. Alumina cream alone is used in the clarification, thus avoiding the danger of heating the solution to a high temperature in the presence of an excess of lead acetate.

An interesting fact is observed in cooling solutions of honey to 0° . The maximum left-hand rotation is not reached as soon as the temperature reaches 0° but only after it has been kept at that temperature for two or three hours. The line representing the change in rotatory power in solutions of honey between 10° and 88° is practically straight but from 10° to 0° , if measured by the readings taken without delay, it is decidedly curved; the reading being less at first than it is afterwards. After three hours the 0° becomes sensibly constant and then the whole line is nearly straight, but still with a slight deficiency in the reading at the 0° . For this reason the computations should be based on readings between 10° and 88° rather than on a number covering the whole range of temperature. Nevertheless, if the solution be kept at 10° for three hours before the final reading is taken, no error of any practical magnitude is introduced.

The calculations given above, for the cane sugar scale, can also be made in an exactly similar manner for angular rotation. The angular variation produced by one gram of levulose for 1° of temperature is $0^{\circ}.01256$. For 88° this would become $1^{\circ}.10528$. Suppose the total observed angular deviation in a given case between 0° and 88° to be $10^{\circ}.404$, then the weight of levulose present is $10.404 \div 1.10528 = 9.413$ grams.

In the case mentioned 26.048 grams of honey were taken for the examination. The percentage of levulose present, therefore, was $9.413 \times 100 \div 26.048 = 36.13$.

261. General Formula for the Calculation of Percentage of Levulose.—Let K = deviation in divisions of the cane sugar scale or in angular rotation produced by one gram of levulose for 1 temperature.

Let T and t' = temperatures at which observations are made.

Let R = observed deviation in rotation.

Let W = weight of levulose obtained.

Let L = per cent. of levulose required.

$$\text{Then} \quad L = \frac{R}{K(T-t')} \div W.$$

In most genuine honeys the value of R between 0° and 88° is approximately thirty divisions of the cane sugar scale or 10° angular measure for 26.048 grams in 100 cubic centimeters, read in a 200 millimeter tube, or, for 13.024 grams in 100 cubic centimeters read in a 400 millimeter tube.

The method of analysis outlined above has been applied in the examination of a large number of honeys with most satisfactory results. It can also be applied with equal facility to other substances containing levulose. In the above calculations the base weight 26.048 grams and the 100 cubic centimeter flask are the figures used with the ventzke scale.

262. Sucrose and Dextrose.—In mixtures these two sugars are easily determined by optical processes, provided no other bodies sensibly affecting the plane of polarized light be present. The total deviation due to both sugars is determined in the usual way. The percentage of sucrose is afterwards found by the inversion method (126). The rotation, in the first instance due to the sucrose, is calculated from the amount of this body found by inversion, and the residual rotation is caused by the dextrose. The percentage of dextrose is easily calculated by a simple proportion into which the numbers expressing the gyrodyne of sucrose and dextrose enter. When the readings are made on a ventzke scale the calculations are made as follows:

	Grams.
Weight of sample used.....	26.048
First polarization	$88^\circ.5$
Polarization after inversion	$10^\circ.5$
Temperature	$20^\circ.0$
Percentage of sucrose.....	58.4
Rotation due to dextrose.....	$30^\circ.1$

Percentage of dextrose:

$$66.5 : 53 = x : 30.1; \text{ whence } x = 37.8.$$

The sample examined therefore contains 58.4 per cent. of sucrose and 37.8 per cent. of dextrose.

It is evident that the method just described is also applicable when maltose, dextrin, or any other sugar or polarizing body, not sensibly affected by the process of inversion to which the sucrose is subjected, is substituted for dextrose. When, however, more than two optically active bodies are present the purely polariscopic process is not applicable. In such cases the chemical or the combined chemical and optical methods described further on can be employed.

263. Lactose in Milk.—By reason of its definite gyrodynic lactose in milk is quickly and accurately determined by optical methods, when proper clarifying reagents are used to free the fluid of fat and nitrogenous substances. Soluble albuminoids have definite levogyrotory powers and, if not entirely removed, serve to diminish the rotation due to the lactose.

Milk casein precipitated by magnesium sulfate has the following rotatory numbers assigned to it:⁸⁰

Dissolved in water $[\alpha]_D = -80^\circ$.

“ “ very dilute solution $[\alpha]_D = -87^\circ$.

“ “ dilute sodium hydroxid solution $[\alpha]_D = -75^\circ$.

“ “ strong potassium hydroxid solution $[\alpha]_D = -91^\circ$.

The hydrates of albumen have rotation powers which vary from $[\alpha]_D = -71^\circ.40$ to $[\alpha]_D = -79^\circ.05$. From the chaotic state of knowledge concerning the specific rotating power of the various albumens, it is impossible to assign any number which will bear the test of criticism. For the present, however, this number may be fixed at $[\alpha]_D = -70^\circ$ for the albumens which remain in solution in the liquids polarized for milk sugar.⁸¹

Many reagents have been prepared for the removal of the disturbing bodies from milk in order to make its polarization pos-

⁸⁰ Hoppe-Seyler, *Handbuch der Physiologisch-und Pathologisch-Chemischen Analyse*, 8th Edition, 1909 : 489.

⁸¹ Kühne und Chittenden, *American Chemical Journal*, 1884-85, 6 : 45.

sible. Among the precipitants which have been used in this country may be mentioned:⁸²

- (1) Saturated solution basic lead acetate, specific gravity 1.97:
- (2) Nitric acid solution of mercuric nitrate diluted with an equal volume of water:
- (3) Acetic acid, specific gravity 1.040, containing 29 per cent. acetic acid:
- (4) Nitric acid, specific gravity 1.197, containing 30 per cent. nitric acid:
- (5) Sulfuric acid, specific gravity 1.255, containing 31 per cent. sulfuric acid:
- (6) Saturated solution of sodium chlorid:
- (7) Saturated solution of magnesium sulfate:
- (8) Solution of mercuric iodid in acetic acid, formula; potassium iodid, 33.2 grams; mercuric chlorid, 13.5 grams; strong acetic acid, 20.0 cubic centimeters; water 640 cubic centimeters.

Alcohol, ether, and many solutions of mineral salts, hydrochloric and other acids are also used as precipitants for albumen, but none of them presents any advantages.

Experience has shown that the best results in polariscopic work are secured by the use of either the mercuric iodid or the acid mercuric nitrate for clarifying the milk. The latter reagent should be used in quantities of about three cubic centimeters for each 100 of milk. It is evident when it is desired to determine the residual nitrogen in solution, the former reagent must be employed. The quantity of albuminoid matter left in solution after clarification with mercurial salts is so minute as to exert no sensible effect on the rotation of the plane of polarized light produced by the lactose.

For purposes of calculation the gyrodyn of lactose in the ordinary conditions of temperature and concentration may be represented by $[\alpha]_D = 52^\circ.5$.

Polarization.—The proper weight of milk is placed in a sugar flask, diluted with water, clarified with the mercuric salt, the volume completed to the mark, and the contents shaken and

⁸² Kühne und Chittenden, American Chemical Journal, 1884-85, 6 : 289.

poured on a filter. The filtrate is polarized in tubes of convenient length. The observed rotation may be expressed either in degrees of angular measurement or of the sugar scale. The weight of milk used may be two or three times that of the normal weight calculated for the instrument employed. Instead of weighing the milk a corresponding volume determined by its specific gravity may be delivered from a burette-pipette (page 308). For the laurent polariscope three times, and for the half-shadow instruments for lamplight, twice the normal weight of milk should be used. For approximately 60 cubic centimeters of milk the flask should be marked at 105 cubic centimeters in compensation for the volume of precipitated solids or the reading obtained from a 100 cubic centimeter flask, decreased by one-twentieth.

For the laurent instrument the normal weight of lactose is determined by the following proportions:

Gyrodynic of sucrose, 66.5 : lactose : 52.5 = x : 16.19.

Whence $x = 20.51$, that is, the number of grams of pure lactose in 100 cubic centimeters required to read 100 divisions of the sugar scale of the instrument.

For the ventzke scale the normal quantity of lactose required to read 100 divisions is found from the following equation:

$$66.4 : 52.5 = x : 26.048$$

Whence $x = 32.74$.

In the one case three times the normal weight of milk is 61.53 and in the other twice the normal weight, 65.48 grams.

264. Error due to Volume of Precipitate.—Vieth states that the volume allowed for the precipitated solids in the original process, *viz.*, two and four-tenths cubic centimeters, is not sufficiently large.⁸³ In such cases it is quite difficult to decide on any arbitrary correction based on the supposed quantities of fat and albuminoids present. A better method than to try to compensate for any arbitrary volume is to remove entirely the disturbing cause or eliminate it by indirect means. To wash the precipitate free of sugar without increasing the bulk of the filtrate unduly would be extremely difficult and tend, moreover, to bring some of

⁸³ The Analyst, 1888, 13 : 64.

the precipitated matters again into solution. It is better, therefore, to eliminate the error by double dilution and polarization. The principle of this method is based on the fact, that within limits not sensibly affecting the gyrodyne by reason of different densities, the polarizations of two solutions of the same substance are inversely proportional to their volumes.

For convenience, it is recommended that the volumes of the samples in each instance be 100 and 200 cubic centimeters, respectively, in which case the true reading is obtained by a simple formula.⁸⁴

265. Sucrose and Raffinose.—In raw sugars made from beet molasses considerable quantities of raffinose are found. The method of inversion, polarization and calculation, in such cases has already been discussed.

SEPARATION OF SUGARS BY CHEMICAL AND CHEMICAL- OPTICAL METHODS.

266. Conditions of Separation.—In the foregoing paragraphs the optical methods for determining certain sugars have been described. Many cases arise, however, in which these processes are inapplicable or insufficient. In these instances, the analyst, as a rule, will be able to solve the problem presented by the purely chemical methods which have been previously described, or by a combination of the chemical and optical processes. Not only have the different sugars distinctive relations to polarized light, but also they are oxidized by varying quantities of metallic salts and these differences are sufficiently pronounced to secure in nearly every instance, no matter how complex, data of a high degree of accuracy.

The carbohydrates of chief importance, from an agricultural point of view, are starch and sucrose; while the alteration products of chief importance, derived therefrom by chemical and biological means, are dextrin, maltose, dextrose and invert sugar.

⁸⁴ Journal American Chemical Society, 1896, 18 : 438.

267. Commercial Glucose and Starch Sugar.—The commercial products obtained by the hydrolysis of starch are known in the trade as glucose and starch sugar. The former term is applied to the thick sirup obtained by concentrating the products of a partial hydrolysis, while the latter is applied to the solid semi-crystalline mass, secured by continuing the hydrolyzing action until the intermediate products are almost completely changed to dextrose. In this country the starch employed is obtained almost exclusively from maize, and the hydrolyzing agent used is hydrochloric acid. The products of conversion in glucose are chiefly dextrins and dextrose with some maltose, and in starch sugar almost entirely dextrose. When diastase is substituted for an acid, as the hydrolytic agent, maltose is the chief product, the ferment having no power of producing dextrose. In the glucose of Japan, known as midzu ame dextrin and maltose are the chief constituents.⁸⁵

Commercial glucose is used chiefly by confectioners, for manufacturing table sirups and for adulterating honey and molasses.

Commercial grape sugar is chiefly employed by brewers as a substitute for barley and other grains.

In Europe, the starch which is converted into glucose, is derived principally from potatoes. The method employed in conversion, whether an acid or diastatic action, is revealed not only by the nature of the product, but also by the composition of its ash. In the case of diastatic conversion the ash of the sample will contain only a trace of sulfates, no chlorin, and be strongly alkaline, while the products of conversion with sulfuric acid will give an ash rich in sulfates with a little lime and be less strongly alkaline.

The process of manufacture in this country consists in treating the starch, beaten to a cream with water, with hydrochloric acid, usually under steam pressure, until the product shows no blue color with iodine. The excess of acid is removed with sodium carbonate, the sirup separated by filtration, formerly whitened by bleaching with sulfurous acid, but now chiefly by passing it

⁸⁵ Wiley and McElroy, *Agricultural Science*, 1892, 6 : 57.

through bone-black, and evaporated to the proper consistence in a vacuum. The solid sugar, consisting mostly of dextrose, is made in the same manner, save that the heating with the acid is continued until the dextrin and maltose are changed into dextrose. The product is either obtained in its ordinary hydrated form or by a special method of crystallization secured as bright anhydrous crystals. Solutions of dextrose, when first made, show mutarotation, but attain their normal gyrodyneic state on standing for 24 hours in the cold, or immediately on boiling.

268. Methods of Separation.—The accurate determination of the quantities of the several optically active bodies formed in commercial glucose is not possible by any of the methods now known. Approximately accurate data may be secured by a large number of processes, and these are based chiefly on the ascertainment of the rotation and reducing power of the mixed sugars, the subsequent removal of the dextrose and maltose by fermentation or oxidation and the final polarization of the residue. The difficulties which attend these processes are alike in all cases. Fermentation may not entirely remove the reducing sugars or may act slightly on the dextrin. In like manner the oxidation of these sugars by metallic salts may not entirely decompose them, may leave an optically active residue, or may affect the optical activity of the residual dextrin. The quantitative methods of separating these sugars by means of phenylhydrazin, lead salts or earthy bases have not been developed into reliable and applicable laboratory processes. At the present time the analyst must be contented with processes confessedly imperfect, but which, with proper precautions, yield data which are nearly correct. The leading methods depending on fermentation and oxidation combined with polarimetric observations will be described in the subjoined paragraphs.

269. Fermentation Method.—This process is based on the assumption that, under certain conditions, dextrose and maltose may be removed from a solution and the dextrin be left unchanged. In practice, approximately accurate results are obtained by this method, although the assumed conditions are not

strictly realized. In the prosecution of this method the polarimetric reading of the mixed sugars is made, and the maltose and dextrose removed therefrom by fermentation with compressed yeast. The residual dextrins are determined by the polariscope on the assumption that their average gyrodyne is 193. In the calculation of the quantities of dextrose and maltose their gyrodynes are fixed at 53 and 138 respectively. The total quantity of reducing sugar is determined by the usual processes. The relative reducing powers of dextrose and maltose are represented by 100 and 62 respectively. The calculations are made by the following formulas:⁸⁶

R = reducing sugars as dextrose

d = dextrose

m = maltose

d' = dextrin

P = total polarization (calculated as apparent gyrodyne)

P' = rotation after fermentation (calculated as apparent gyrodyne).

$$\text{Whence } R = d + 0.62 m \dots \dots \dots (1)$$

$$P = 53 d + 138 m + 193 d' \dots \dots (2)$$

$$P' = 193 d' \dots \dots \dots (3)$$

From these three equations the values of d , m , and d' are readily calculated:

Example: To find d and m :

$$\text{Subtract (3) from (2) } P = 53d + 138m + 193d'$$

$$\begin{array}{r} P' = 193d' \\ \hline P - P' = 53d + 138m \dots \dots \dots (4) \end{array}$$

Multiply (1) by 53 and subtract from (4)

$$\begin{array}{r} P - P' = 53d + 138m \\ 53R = 53d + 32.86m \\ \hline P - P' - 53R = 105.14m \dots \dots \dots (5) \\ (P - P' - 53R) \end{array}$$

$$\text{Whence } m = \frac{(P - P' - 53R)}{(105.14)} \dots \dots \dots (6)$$

$$d = R - 0.62m \dots \dots \dots (7)$$

$$d' = \frac{P'}{193} \dots \dots \dots (8)$$

⁸⁶ Wiley, Chemical News, 1882, 46 : 175.

Sidersky assigns the values $[\alpha]_D = 138.3$ and $[\alpha]_D = 194.8$ to maltose and dextrin respectively in the above formulas.⁸⁷

Illustration: In the examination of a sample, 26.048 grams, of midzu ame in 100 cubic centimeters polarized in a 200 millimeter tube gave the following data.

Polarization of sample in angular degrees $69^{\circ}.06$, which is equal to an apparent gyrodyne of 132.6:

Total reducing sugar as dextrose 33.33 per cent:

Polarization in angular degrees after fermentation $30^{\circ}.84$
 $[\alpha]_D = 59.2$.

Substituting these values in the several equations gives the following numbers:

- (1) $0.3333 = d + 0.62 m$
- (2) $132.6 = 53 d + 138 m$
- (3) $59.2 = 193 d'$
- (4) $73.4 = 53 d' + 138 m$
- (5) $55.74 = 105.14 m$
- (6) $m = 0.5301 = 53.01$ per cent.
- (7) $d = 3333 - 3286 = 0.0047 = 0.47$ per cent.
- (8) $d' = 59.2 \div 193 = 0.3067 = 30.67$ per cent.

Summary: Sample of midzu ame:

	Per cent.
Percentage of dextrin	30.67
“ “ maltose	53.01
“ “ dextrose	00.47
“ “ water	14.61
“ “ ash	00.31
Sum	99.07
Undetermined	0.93

For polarization the lamplight shadow polariscope employed for sugar may be used, and the degrees of the sugar (ventzke) scale converted into angular degrees by multiplying by 0.3467.

The process of fermentation is conducted as described in a paragraph given further on, relating to the determination of lactose in the presence of sucrose.

⁸⁷ Sidersky, *Traité d'Analyse des Matières Sucrées*, 1890 : 352.

270. The Oxidation Method.—The removal of the reducing sugars may be accomplished by oxidation instead of fermentation. The process of analysis is in all respects similar to that described in the foregoing paragraph, substituting oxidation for fermentation.⁸⁸ For the oxidizing agent mercuric cyanid is preferred, and it is conveniently prepared by dissolving 120 grams of mercuric cyanid and an equal quantity of sodium hydroxid in water mixing the solutions and completing the volume to one liter. If a precipitate be formed in mixing the solutions it should be removed by filtering through asbestos. For the polarization, 10 grams of the sugars in 100 cubic centimeters is a convenient quantity. Ten cubic centimeters of this solution are placed in a flask of water marked at 50 cubic centimeters, a sufficient quantity of the mercuric cyanid added to remain in slight excess after the oxidation is finished (from 20 to 25 cubic centimeters) and the mixture heated to the boiling point for three minutes. The alkali, after cooling, is neutralized with strong hydrochloric acid and the passing from alkalinity to acidity will be indicated by a discharge of the brown color which is produced by heating with the alkaline mercuric cyanid. The heating with the mercury salt should be conducted in a well ventilated fume chamber.

The calculation of the results is conducted by means of the formulas given in the preceding paragraph. In the original paper describing this method, it was stated that its accuracy depended on the complete oxidation of the reducing sugar in a manner leaving no optically active products, and on the inactivity of the reagents used in respect to the dextrin present. These two conditions are not rigidly fulfilled, as is shown by Wilson.⁸⁹ According to his data maltose leaves an optically active residue, which gives a somewhat greater right hand rotation than is compensated for by the diminished rotation of the dextrin. Wilson, however, confesses that the dextrin used contained reducing sugars, which would not be the case had it been prepared by the process of treating it with alkaline mercuric cyanid as above indicated. Upon

⁸⁸ Wiley, Chemical News, 1882, 46 : 175.

⁸⁹ Chemical News, 1892, 65 : 169.

the whole, the oxidation of the reducing sugar by a mercury salt gives results which, while not strictly accurate, are probably as reliable as those afforded by fermentation.

271. Removal of Dextrose by Copper Acetate.—Maercker first called attention to the fact that Barfoed's reagent (one part copper acetate in 15 parts of water, and 200 cubic centimeters of this solution mixed with five cubic centimeters of 38 per cent. acetic acid) reacts readily with dextrose, while it is indifferent to maltose and dextrans. Sieben's method of removing dextrose is based on this fact.⁹⁰ It is found that under certain conditions pure maltose does not reduce either the acidified or neutral solution of copper acetate, while dextrose or a mixture of dextrose and maltose does so readily. It is also shown that the fermentation residue under suitable conditions acts like maltose. Maltose solutions reduce the reagent after boiling four minutes while at 40°-45° they have no effect even after standing four days. The amount of copper deposited by dextrose, under the latter conditions, is found to depend to a certain extent on the amount of free acetic acid present, and as the solutions of copper acetate always contain varying quantities of acetic acid which cannot be removed without decomposition and precipitation of basic salt, the use of an absolutely neutral solution is impracticable. The reagent prepared according to Barfoed's directions is almost saturated, but a half normal solution is preferable. Sieben proposes two solutions: I, containing 15.86 grams copper and 0.56 gram acetic anhydrid per liter; II, containing 15.86 grams copper and three grams acetic anhydrid per liter. The reduction of the dextrose is secured by placing 100 cubic centimeters of the solution in a bottle, adding the sugar solution, stoppering and keeping in a water-bath at 40°-45° two or three days. An aliquot portion is then drawn off and the residual copper precipitated by boiling with 45 cubic centimeters of alkali solution of the Fehling reagent and 40 cubic centimeters of one per cent. dextrose solution, filtered and weighed as usual. The results show that either

⁹⁰ Zeitschrift des Vereins für die Rübenzucker-Industrie, 1884, 34, neuer folge 21 : 854.

solution can be used, and that standing for two days at 45° is sufficient. One hundred cubic centimeters of the copper solution are mixed with 10 cubic centimeters of the sugar solution containing from two-tenths to five-tenths gram of dextrose, as this dilution gives the best results. No reduction is found to have taken place when solutions containing five-tenths gram of maltose or five-tenths gram fermentation residue are used. The data can not be compiled in the form of a table similar to Allihn's as it is impossible to obtain a solution of uniform acidity each time, and the solution will have to be standardized by means of a known pure dextrose solution and the result obtained with the unknown sugar solution properly diluted compared with this. This method of Sieben's has never been practiced to any extent in analytical separations and can not, therefore, be strongly recommended without additional experience. Hinkel and Shearman have made a careful study of this method and found that the test requires very careful regulation as to details of manipulation and amount of sugar tested, but under such restrictions is capable of greater usefulness than has generally been appreciated.⁹¹

272. Analysis of Sugar Mixtures.—Browne has reviewed the principal methods of separating sugar mixtures and subjected the more promising processes to experimental proof.⁹² The most important factor in these separations is the copper reducing power of the mixture. Browne determines all the reducing sugars present as dextrose. This procedure is justified by the statement that the use of a constant ratio established in terms of dextrose for each sugar the percentages of the different constituents in a mixture can be easily calculated. The next most useful factor in these separations is the rotating power of the sum of the sugars.

273. Application of the Methods.—In the case of mixtures containing two reducing sugars the calculations are made from determinations of the reducing power and polarization.

⁹¹ Journal American Chemical Society, 1907, **29** : 1744-7.

⁹² Journal of the American Chemical Society, 1906, **38** : 441.

Let x = per cent. of a given sugar A ;

y = " " " " " " B ;

a = dextrose ratio of sugar A ;

b = " " " " " B ;

R = per cent. of total reducing sugars as dextrose ;

then I, $ax + by = R$.

The polarization of a mixture of sugars is equal to the sum of the polarizations of the individual sugars present. As the S. & H. type of saccharimeter is the form of polariscope most commonly used, all polarizations were made with this instrument. 26.048 grams of material being taken for analysis, and the readings made in a 200 millimeter tube. The polarization (Ventzke) of any sugar is equal to its percentage multiplied by a polarization factor, found by dividing the specific rotation of the sugar by the specific rotation of sucrose (+66.5).

Let a = polarization factor of sugar A ;

β = " " " " " B ;

P = polarization (Ventzke) of mixture ;

then II, $ax + \beta y = P$.

From formulas I and II we obtain

$$x = \frac{bP - \beta R}{a\beta - ab'}$$

and

$$y = \frac{R - ax}{b}$$

The above formulas may be applied to the analysis of known mixtures of the common reducing sugars, dextrose, levulose, galactose, xylose and arabinose.

274. Separation of Levulose and Dextrose.—In this separation the dextrose ratio of levulose = 0.915 is used.

The polarization factor of levulose (20°C. 10 per cent. solution) =

$$\frac{-90.18}{+66.5} = -1.356.$$

The polarization factor of dextrose = $\frac{+52.74}{+66.5} = 0.793$.

Owing to the great susceptibility of levulose to variations in specific rotation through changes of temperature and concentration, the use of a fixed polarization factor is only possible when the analyses are made under perfectly similar conditions. The

value of the polarization factor of levulose for different temperatures and concentrations is given in the following table:

Temp. Degrees.	Concentration.						
	1 per cent.	2 per cent.	3 per cent.	4 per cent.	5 per cent.	10 per cent.	25 per cent.
15	-1.384	1.385	-1.387	-1.389	-1.390	-1.398	-1.422
20	-1.341	-1.343	-1.345	-1.346	-1.348	-1.356	-1.380
25	-1.299	-1.301	-1.303	-1.304	-1.306	-1.314	-1.338
30	-1.257	-1.259	-1.261	1.262	-1.264	-1.272	-1.296

The above figures were calculated from the general formula of Jungfleisch and Grimbert, $[\alpha]_D = (101.38 - 0.56t + 0.108(t-10))$.

The variations of the polarization constant due to concentration are so small that they do not affect the accuracy of the calculations appreciably and a 10 per cent. concentration was taken as the basis. The influence of temperature, however, is so pronounced that it cannot be disregarded.

The following formulas were employed in the analysis of levulose and dextrose mixtures:

$$\text{I. Per cent. levulose (L)}_{20} = \frac{0.793R - P}{2.08}$$

$$\text{II. Per cent. dextrose} = R - 0.915 \text{ L.}$$

For other temperatures than 20° the value of the denominator in equation I is 2.12 (15°), 2.04 (25°), 2.00 (30°).

In the following table are given the analyses of seven mixtures containing known amounts of levulose and dextrose.

Taken		R	P°.	Found		Error	
Levulose Per cent	Dextrose Per cent			Levulose Per cent	Dextrose Per cent	Levulose Per cent	Dextrose Per cent
0.99	2.06	3.01	0.35 22°	0.98	2.11	-0.01	0.05
1.59	5.92	7.41	2.65 23°	1.56	5.98	0.03	0.06
3.17	11.83	14.94	5.30 23°	3.02	11.78	-0.15	0.05
4.52	4.84	9.06	-2.15 22°	4.51	4.83	-0.01	0.01
5.63	1.85	7.02	-6.00 23°	5.61	1.89	-0.02	0.04
9.04	9.67	17.80	-4.30 22°	8.90	9.66	-0.14	0.01
11.26	3.69	14.94	12.00 23°	11.23	3.76	-0.03	0.07
Average error,						-0.06	0.04

The percentage of invert sugar in mixtures of dextrose and levulose is easily found by combining the smaller percentage with an equal amount of the other component. The dextrose ratio of invert sugar is $\frac{1.915}{2} = 0.958$, and since 100 parts of invert

sugar correspond to 95 of sucrose, the dextrose equivalent of an inverted sucrose solution gives almost exactly the amount of sucrose. One part of dextrose = $\frac{0.95}{0.958} = 0.993$ part sucrose.

275. Separation of Dextrose and Galactose.—In this separation the dextrose ratio of galactose = 0.898 is used.

The polarization factor of galactose (20°, 10 per cent solution)

$$\frac{+80.49}{+66.5} = 1.21.$$

The specific rotation of galactose varies somewhat with temperature and concentration, the differences, however, being much less than those of levulose. The following values for the polarization factor of galactose at different temperatures and concentrations were calculated from the general formula of Meissl.⁹³

$$[\alpha]_D^t = 83.037 + 0.0785 t - 0.209 t^2.$$

Temperature Degrees	10 per cent	15 per cent	20 per cent
10	1.242	1.248	1.254
20	1.210	1.216	1.222
30	1.179	1.185	1.191

The concentration influence of galactose upon the polarization factor is too slight to influence the calculations appreciably; the temperature influence, however, should be regarded in case the readings are made very much above or below 20°.

The following formulas were employed in making the separations of dextrose and galactose:

$$\text{I. Per cent. dextrose (D)}_{20^\circ} = \frac{1.21 R - 0.898 P}{0.498},$$

$$\text{II. Per cent. galactose} = \frac{R - D}{0.898}$$

$$\text{At } 25^\circ \text{ formula I would be } D = \frac{1.195 R - 0.898 P}{0.482}.$$

In the following table are given the analyses of 4 mixtures containing known amounts of dextrose and galactose.

Taken				Found		Error		
Dextrose Per cent.	Galactose Per cent.	R	P ⁹³	Dextrose Per cent.	Galactose Per cent.	Dextrose Per cent.	Galactose Per cent.	
2.12	7.68	9.06	+11.0 25°	1.97	7.89	-0.15	+0.21	
4.24	15.35	18.16	+21.9 25°	4.23	15.51	-0.01	+0.16	
5.15	2.34	9.29	+8.5 25°	7.20	2.33	+0.05	-0.01	
11.29	4.68	18.35	+17.0 25°	13.82	5.04	-0.47	+0.34	

Average error, ± 0.17 ± 0.18

⁹³ Journal für praktische Chemie [2], 22 : 97.

The average error in the above series of experiments is nearly four times that found in the separation of levulose and dextrose. This was to be expected since, owing to the small difference in the specific rotations of dextrose and galactose, the errors of observation are doubled; in the analysis of the dextrose-levulose mixtures on the other hand the wide range in the specific rotation diminishes the experimental errors one-half (compare Formula I for both separations).

276. Separation of Levulose and Galactose.—In making this separation the following formulas were employed:

$$\text{I. Per cent. levulose } (L)_{20^\circ} = \frac{1.21 R - 0.898 P}{2.324},$$

$$\text{II. Per cent. galactose} = \frac{R - 0.915 L}{0.898}.$$

The susceptibility of the specific rotations of both levulose and galactose to temperature variations necessitates a considerable temperature correction if the readings are not made at 20°. This can be done by using the polarization factors given above.

III and V. At 30°, *e. g.*, formula I would be $L =$

$$\frac{1179R - 0.898 P}{2.221}.$$

In the following table are given the analyses of 4 mixtures containing known amounts of levulose and galactose.

Taken				Found				Error	
Levulose Per cent.	Galactose Per cent.	R	P^{40}	Levulose Per cent.	Galactose Per cent.	Levulose Per cent.	Galactose Per cent.	Levulose Per cent.	Galactose Per cent.
1.24	8.56	8.78	+ 8.75 28°	1.14	8.62	+0.10	-0.06		
2.47	17.12	17.78	+17.40 25°	2.46	17.29	-0.01	+0.17		
5.44	1.40	6.11	- 5.35 28°	5.38	1.33	-0.06	-0.07		
10.89	2.80	12.31	-10.50 29°	10.76	2.74	-0.13	-0.06		

Average error, ± 0.07 ± 0.09

277. Separation of Levulose and Arabinose.—Here the dextrose ratio of arabinose = 1.032 is used.

$$\text{Polarization factor of arabinose } (20^\circ) = \frac{104.5}{66.5} = 1.571.$$

The formulas employed for the separation of levulose and arabinose are the following:

$$\text{I. Per cent. levulose } (L)_{20^\circ} = \frac{1.571 R - 1.032 P}{2.836},$$

$$\text{II. Per cent. arabinose} = \frac{R - 0.915 L}{1.032}$$

The specific rotation of arabinose varies somewhat with changes in temperature and concentration, but these variations are not great enough to seriously affect the calculations. The temperature corrections for levulose, however, must be made as usual. The denominator in equation I becomes 2.88 at 15°, 2.793 at 25°, and 2.75 at 30°.

In the following table are given the analyses of two mixtures containing known amounts of levulose and arabinose.

Taken				Found		Error	
Levulose Per cent.	Arabinose Per cent.	R	P ¹⁰	Levulose Per cent.	Arabinose Per cent.	Levulose Per cent.	Arabinose Per cent.
7.41	2.28	9.05	— 6.1 27°	7.39	2.22	—0.02	—0.06
14.82	4.55	18.14	—12.3 26°	14.80	4.46	0.02	0.09
Average error,						—0.02	—0.07

In the estimation of levulose and arabinose we have a wider range of specific rotations than with any other mixture of two sugars and a corresponding reduction in the experimental sources of error.

278. Separation of Xylose and Arabinose.—In this process the dextrose ratio of xylose = 0.983.

and the polarization factor of xylose = $\frac{+18.79}{+66.5} = 0.283$.

The formulas employed for the separation of xylose and arabinose are the following:

$$\text{I. Per cent. xylose (X)} = \frac{1.571 R - 1.032 P}{1.252},$$

$$\text{II. Per cent. arabinose} = \frac{R - 0.983 X}{1.032}.$$

In the following table are given the analyses of four mixtures containing known amounts of xylose and arabinose.

Taken				Found		Error	
Xylose Per cent.	Arabinose Per cent.	R	P ¹⁰	Xylose Per cent.	Arabinose Per cent.	Xylose Per cent.	Arabinose Per cent.
1.98	6.14	8.35	+10.2 25°	2.05	6.13	+0.07	—0.01
3.96	12.28	16.66	+20.3 25°	4.17	12.17	+0.21	—0.11
6.05	1.73	7.85	+4.5 25°	6.14	1.75	+0.09	+0.02
12.10	3.46	15.46	+8.8 25°	12.14	3.42	+0.04	—0.04
Average error,						+0.10	±0.05

279. The Determination of Dextrose, Levulose and Sucrose in Mixtures.—The analysis of a mixture of dextrose, levulose and sucrose is a problem which frequently confronts the sugar

chemist. The polarization of a mixture of the three sugars is expressed by the formula

$$S + 0.793D - 1.356L = P_{20^{\circ}}.$$

Substituting the above equation in the formulas employed for the separation of dextrose and levulose alone, we obtain, letting S equal the per cent. sucrose by Clerget:

$$\text{I. Per cent. levulose } (L)_{20^{\circ}} = \frac{0.793 R + S - P}{2.08},$$

$$\text{II. Per cent. dextrose } = R - 0.915 L.$$

280. Sources of Error.—Brown found out two sources of error in carrying out the analytical work outlined above *viz.*, the slight reducing action of sucrose on the Fehling solution and second the change in the rotation of levulose in neutral and acid solution. When much levulose is present the hydrochloric acid used in the inversion of the sucrose will so alter the rotation of levulose as to increase the apparent quantity of sucrose present. With quantities of levulose equal to 15 per cent. of the sample the quantity of sucrose obtained will be about half a per cent. too high.

281. Removal of Dextrin by Alcohol.—By reason of its less solubility, dextrin can be removed from a solution containing also dextrose and maltose by precipitation with alcohol. It is impracticable, however, to secure always that degree of alcoholic concentration which will cause the coagulation of all the dextrans without attacking the concomitant reducing sugars. In my experience it has been found impossible to prepare a dextrin by alcoholic precipitation, which did not contain bodies capable of oxidizing alkaline copper solutions.

The solution containing the dextrin is brought to a sirupy consistence by evaporation and treated with about 10 volumes of 90 per cent. alcohol. After thorough mixing, the precipitated dextrin is collected on a filter and well washed with alcohol of the strength noted. It is then dried and weighed. If weaker solutions of dextrin are used, the alcohol must be of correspondingly greater strength. In the filtrate the residual maltose and dextrose may be separated and determined by the chemical and optical methods already described. Browne has

made a report on the assay of commercial dextrans which may be profitably studied in this connection.⁹⁴

CARBOHYDRATES IN MILK.

282. Milk Sugar (Lactose).—(a) OPTICAL METHOD—OFFICIAL.⁹⁵—(1) PREPARATION OF REAGENTS.—(a) *Acid Mercuric Nitrate*.—Dissolve mercury in double its weight of nitric acid, specific gravity 1.42, and dilute with an equal volume of water. One cubic centimeter of this reagent is sufficient for the quantities of milk mentioned below. Larger quantities may be used without affecting the results of polarization.

(b) *Mercuric Iodid with Acetic Acid*.—Mix 33.2 grams of potassium iodid, 13.5 grams of mercuric chlorid, 20 cubic centimeters of glacial acetic acid, and 640 cubic centimeters of water.

(2) DETERMINATION.—The milk should be at a constant temperature, and its specific gravity determined with a delicate hydrometer. When greater accuracy is required, a pycnometer is used.

The quantities of the milk measured for polarization vary with the specific gravity of the milk as well as with the polariscope used. The quantity to be measured in any case will be found in the following table:

DETERMINATION OF VOLUME OF MILK SAMPLE.

Specific gravity	Volume of milk to be used	
	For polariscopes of which the sucrose normal weight is 16.19 grams. cc	For polariscopes of which the sucrose normal weight is 26.048 grams. cc
1.024	60.0	64.4
1.026	59.9	64.3
1.028	59.8	64.15
1.030	59.7	64.0
1.032	59.6	63.9
1.034	59.5	63.8
1.035	59.35	63.7

Place the quantity of milk indicated in the table in a flask graduated at 102.4 cubic centimeters for a Laurent or 102.6 cubic centimeters for a Ventzke polariscope (Mohr cubic centimeter).

⁹⁴ Bureau of Chemistry, Bulletin 116, 1908 : 66.

⁹⁵ Bureau of Chemistry, Bulletin 107, revised, 1912 : 118.

Add 1 cubic centimeter of mercuric nitrate solution or 30 cubic centimeters of mercuric iodid solution (an excess of these reagents does no harm), fill to the mark, agitate, filter through a dry filter, and polarize. It is not necessary to heat before polarizing. In case a 200 millimeter tube is used, divide the polariscope reading by 3 when the sucrose normal weight for the instrument is 16.79 grams, or by 2 when the normal weight for the instrument is 26.048. When a 400 millimeter tube is used, these divisors become 6 and 4, respectively. For the calculation of the above table the specific rotary power of lactose is taken as 52.53° , and the corresponding number for sucrose as 66.5° . The lactose normal weight to read 100° on the sugar scale for Laurent instruments is 20.496 grams, and for Vontzke instruments, 32.975 grams. In case metric flasks are used the normal weights above must be reduced to 16.160 and 26.000 grams respectively.

283. The Official Gravimetric Method.—The alkaline copper method of determining lactose, adopted by the Association of Official Agricultural Chemists, is essentially the procedure proposed by Soxhlet.⁹⁶

(1) **PREPARATION OF THE MILK SOLUTION.**—Dilute 25 cubic centimeters of the milk, held in a half liter flask, with 400 cubic centimeters of water and add 10 cubic centimeters of a solution of copper sulfate of the strength given for Soxhlet's modification of Fehling's solution, page 220; add about seven and a half cubic centimeters of a solution of potassium hydroxid of such strength that one volume of it is just sufficient to completely precipitate the copper as hydroxid from one volume of the solution of copper sulfate. In place of a solution of potassium hydroxid of this strength eight and a half cubic centimeters of a half normal solution of sodium hydroxid may be used. After the addition of the alkali solution the mixture must still have an acid reaction and contain copper in solution. Fill the flask to the mark, shake and filter through a dry filter.

(2) **DETERMINATION.**—Place 50 cubic centimeters of the mixed copper reagent in a beaker and heat to the boiling point. While

⁹⁶ Division of Chemistry, Bulletin 46, revised, 1899 : 41.

boiling briskly, add 100 cubic centimeters of the lactose solution, prepared as directed above, and boil for six minutes. Filter immediately and determine the amount of copper reduced by one of the methods already given, pages 220, 221. Obtain the weight of lactose equivalent to the weight of copper found from the table on pages 221, 222.

FOR DETERMINATION OF SUCROSE IN CONDENSED MILK.

284. Patein et Dufau Reagent.⁹⁷—To 200 grams of yellow HgO, with 400 cubic centimeters H₂O in an evaporator, add cautiously sufficient HNO₃ (about 140 cubic centimeters) to just dissolve the HgO, then add sufficient NaOH solution to make a permanent precipitate, dilute to one liter and filter. As this solution tends to become more acid with age, by the deposition of basic mercury salts, it should receive a little alkali from time to time.

To clarify condensed milk solutions for polariscopic reading.

To 50 cubic centimeters or grams, of either 40 per cent. or 20 per cent. solution of condensed milk, add 25 cubic centimeters of water, 5 cubic centimeters of Patein and Dufau reagent, according to the amount of milk present, and shake well. Without

delay run in, with constant shaking, sufficient $\frac{N}{2}$ NaOH solu-

tion to just bring the reaction to practical neutrality,—but not to alkalinity—using litmus paper as indicator; between 12 and 13 cubic centimeters usually are sufficient. Make up to the 100 cubic centimeter mark, shake well and polarize as usual. Correct for volume of precipitate by use of factor 0.73 per cubic centimeter for protein and 1.075 per cubic centimeter for fat.

Invert the sucrose by HCl in the cold, and proceed as usual for the invert readings.

285. Sugars in Evaporated Milks.—In addition to the lactose normally present in evaporated milks the analyst will, in most cases, find large quantities of sucrose. The latter sugar is added

⁹⁷ *Annales de Chimie Anal.* 1902, 7 : 128.

Zeitschrift für Untersuchung der Nahrungs und Genussmittel 1902, 5 : 726.

as a preservative and condiment. By reason of the ease with which sucrose is hydrolyzed, evaporated milk containing it may have also some invert sugar among its contents. A method of examination is desirable, therefore, which will secure the determination of lactose, sucrose and invert sugar in mixtures. The probability of the development of galactose and dextrose during the evaporation and conservation of the sample, is not great. The best method of conducting this work is the one developed by Bigelow and McElroy.⁹⁸ The principle on which the method is based rests on the fact that in certain conditions, easily supplied, the sucrose and invert sugar present in a sample may be entirely removed by fermentation and the residual lactose secured in an unchanged condition. The lactose is finally estimated by one of the methods already described.

The details of the process follow:

On opening a package of evaporated milk, its entire contents are transferred to a dish and well mixed. Several portions of about 25 grams each are placed in flasks marked at 100 cubic centimeters. To each of the flasks enough water is added to bring all the sugars into solution and normal rotation is made certain by boiling. After cooling, the contents of the flasks are clarified by mercuric iodid in acetic acid solution. The clarifying reagent is prepared by dissolving 53 grams of potassium iodid, 22 grams of mercuric chlorid, and 32 cubic centimeters of strongest acetic acid in water, mixing the solutions and completing the volume to one liter. The clarification is aided by the use of alumina cream (107). The flask is filled to the mark, and the contents well shaken and poured on a filter. After rejecting the first portion of the filtrate the residue is polarized in the usual manner. Two or more separate portions of the sample are dissolved in water in flasks of the size mentioned, heated to 55°, half a cake of compressed yeast added to each and the temperature kept at 55° for five hours. The residue in each flask is treated as above described, the mercuric solution being added before cooling to pre-

⁹⁸ Journal American Chemical Society, 1893, 15 : 668.

vent the fermentative action of the yeast, and the polarization noted.

By this treatment the sucrose is completely inverted, while the lactose is not affected. The percentage of sucrose is calculated by the formulas given in paragraph 132, using the factor 142.6. At the temperature noted the yeast exercises no fermentative, but only a diastatic action.

In each case the volume of the precipitated milk solids is determined by the double dilution method, and the proper correction made (page 139). The lactose remaining is determined by chemical or optical methods, but it is necessary, in all cases where invert sugar is supposed to be present, to determine the total reducing sugars in the original sample as lactose. If the quantity thus determined and the amount of sucrose found as above are sufficient to produce the rotation observed in the first polarization, it is evident that no invert sugar is present. When the polarization observed is less than is equivalent to the quantity of sugar found, invert sugar is present, which tends to diminish the rotation produced by the other sugars. In this case it is necessary to remove both the sucrose and invert sugar by a process of fermentation, which will leave the lactose unchanged.

This is accomplished by conducting the fermentation in the presence of potassium fluorid, which prevents the development of the lactic ferments. For this purpose 350 grams of the evaporated milk are dissolved in water and the solution boiled to secure the normal rotation of the lactose. After cooling to 80°, the casein is thrown down by adding a solution containing about four grams of glacial phosphoric acid and keeping the temperature at 80° for about 15 minutes. After cooling to room temperature, the volume is completed to one liter with water, well shaken and poured onto a filter. An aliquot part of the filtrate is nearly neutralized with a noted volume of potassium hydroxid. Enough water is added to make up, with the volume of potassium hydroxid used, the total space occupied by the precipitated solid, corresponding to that part of the filtrate, and if necessary, re-filter. The volume occupied by the precipitated solids is easily

determined by polarization and double dilution. The filtrate, obtained from the process described above, is placed in portions of 100 cubic centimeters each in 200 cubic centimeter flasks with about 20 milligrams of potassium fluorid in solution, and half a cake of compressed yeast. The yeast is broken up and evenly distributed, and the fermentation is allowed to proceed for 10 days at a temperature of from 25° to 30°. At the end of this time experience has shown that all of the sucrose and invert sugar has disappeared, but the lactose remains intact. The flasks are filled to the mark with water and the lactose determined by chemical or optical methods. By comparing the data obtained from the estimation of the total reducing sugars before fermentation or inversion and the estimation of the lactose after fermentation, the quantity of invert sugar is easily calculated. The experience of many analysts shows that invert sugar is rarely present in evaporated milks, which is an indication that the sucrose added thereto does not generally suffer hydrolysis. The mean percentage of added sucrose found in evaporated milks is about 40.

SEPARATION AND DETERMINATION OF STARCH.

286. Occurrence.—Many bodies containing starch are presented for the consideration of the agricultural analyst. First in importance are the cereals, closely followed by the starchy root crops. Many spices and other condiments also contain starchy matters. In the sap of some plants, for instance sorghum, at certain seasons, considerable quantities of starch occur. In the analysis of cereals and other feeding stuffs, it has been the usual custom to make no separate determination of starch, but to put together all soluble carbohydrates and estimate their percentage by subtracting from 100 the sum of the percentages of the other constituents of the sample. This aggregated mass has been known as nitrogen-free extract. Recent advances in methods of investigation render it advisable to determine the sugars, starch and pentosan carbohydrates separately and to leave among the undetermined bodies the other unclassified substances, chiefly of a carbohydrate nature, soluble in boiling dilute acid and alkali.

287. Separation of Starch.—Starch being insoluble in its natural state, it is impossible to separate it from the other insoluble matters of plants by any known process. In bringing it into solution it undergoes certain changes of an unknown nature, but tending to produce a dextrinoid body. Nevertheless, in order to procure the starch in a state of purity suited to analytical processes, it becomes necessary to separate the starch from the other insoluble bodies that naturally accompany it. As has been shown in preceding paragraphs, there are only two methods of securing the solution of starch which fully meet the conditions of accurate analysis. These are the methods depending on the use of diastatic ferments and on the employment of heat and pressure in the presence of water. These two processes have been described in considerable detail in paragraphs 216-219. It is important, in starch determinations, to remove from the sample the sugar and other substances soluble in water and also the oils, when present in large quantities, before subjecting it to the processes for rendering the starch soluble.

288. Desiccation of Amyliferous Bodies.—The removal of sugars and oils is best secured in amyliferous substances after they are deprived of their moisture. As has already been suggested, the desiccation should be commenced at a low temperature, not above 60° , and continued at that point until the chief part of the water has escaped. The operation may be conducted in one of the ways already described (pp. 14-15). There is great difference of opinion among analysts in respect of the degree of temperature to which the sample should be finally subjected, but for the purposes here in view, it will not be found necessary to go above 105° . Before beginning the operation the sample should be as finely divided as possible, and at its end the dried residue should be ground and passed through a sieve of half a millimeter mesh.

289. Indirect Method of Determining Water in Starch.—It is claimed by Block⁹⁹ that it is necessary to dry starch at 160° in order to get complete dehydration. Wet starch as deposited with its maximum content of water has nine molecules thereof, *viz.*,

⁹⁹ Comptes rendus d l'Academie des Sciences, 118 : 147.

$C_6H_{10}O_5 + 9H_2O$. Ordinary commercial starch has about 18 per cent. of water with a formula of $C_6H_{10}O_5 + 2H_2O$.

The percentage of water may be determined by Block's feculometer or Block's dose-fécule. The first apparatus determines the percentage of anhydrous starch by volume, and the second by weight.

Block's assumption that starch can absorb only 50 per cent. of its weight of water is the basis of the determination.

A noted weight of starch is rubbed up with water until saturated, the water poured off, the starch weighed, dried on blotting paper until it gives off no more moisture and again weighed. Half of the lost weight is water, from which the original per cent. of water can be calculated. This at best seems to be a rough approximation and not suited to rigorous scientific determination.

290. Removal of Oil and Sugar.—The dried, finely powdered sample, obtained as described above, is placed in any convenient extractor (60-78) and the oil or fat it contains removed by the usual solvents. For ordinary purposes, even with cereals, this preliminary extraction of the oil is not necessary, but it becomes so with oily seeds containing starch. The sugar is subsequently removed by extraction with 80 per cent. alcohol and the residue is then ready for the extraction of the starch. In most cases the extraction with alcohol will be found sufficient. In some bodies, for instance the sweet potato (batata), the quantity of sugar present is quite large, and some of it is found in most vegetables. If not present in appreciable amount, the alcohol extraction may also be omitted. The sample having been prepared as indicated, the starch may be brought into solution by one of the methods described in paragraphs 224-226, preference being given to the aqueous digestion in an autoclave. The dissolved starch is washed out of the insoluble residue and determined by optical or chemical methods.

291. Preparation of Diastase for Starch Solution.—The methods of preparing malt extract for use in starch analysis have been

described in paragraph 218. If a purer form of diastase is desired it may be prepared by following the directions given by Long and Baker.¹ Digest 200 grams of ground malt for 24 hours with three parts of 20 per cent. alcohol. Separate the extract by filtration and to the filtrate add about one and a half liters of 93 per cent. alcohol and stir vigorously. After the precipitate has subsided the supernatant alcohol is removed by a syphon, the precipitate is brought onto a filter and washed with alcohol of a strength gradually increasing to anhydrous, and finally with anhydrous ether. The diastase is dried in a vacuum over sulfuric acid and finally reduced to a fine powder before using. Thus prepared, it varies in appearance from a white to a slightly brownish powder. Made at different times and from separate portions of malt, it may show great differences in hydrolytic power.

292. Estimation of Starch in Potatoes by Specific Gravity.—A roughly approximate determination of the quantity of starch in potatoes can be made by determining their specific gravity. Since the specific gravity of pure starch is 1.65, it follows that the richer a potato is in starch the higher will be its specific gravity. The specific weight of substances like potatoes is conveniently determined by suspending them in water by a fine thread attached to the upper hook of a balance pan. There may be a variation of the percentage of other constituents in potatoes as well as of starch, and therefore the data obtained from the following table can only be correct on the assumption that the starch is the only variable. In practice, errors amounting to as much as two per cent. may be easily made, and therefore the method is useful only for agronomic and commercial and not for scientific purposes. The method is especially useful in the selection of potatoes of high starch content for planting. The table is constructed on the weight in grams in pure water of 10,000 grams of potatoes and the corresponding per cents. of dry matter and starch are given. It is not always convenient to use exactly 10,000 grams of potatoes for the determination, but the calculation for any given

¹ Journal of the Chemical Society (Transactions), 1895, 5 : 735.

weight is easy.² More elaborate description of this process are given by Maercker and Delbrück.³

TABLE FOR CALCULATING STARCH IN POTATOES FROM SPECIFIC GRAVITY

10,000 grams of potatoes weigh in water. Grams.	Per cent dry matter.	Per cent starch.	10,000 grams of potatoes weigh in water. Grams.	Per cent dry matter.	Per cent starch.
750	19.9	14.1	1070	28.3	22.5
760	20.1	14.3	1080	28.5	22.7
770	20.3	14.5	1090	28.7	22.9
780	20.7	14.9	1100	29.1	23.3
790	20.9	15.1	1110	29.3	23.5
800	21.2	15.4	1120	29.5	23.7
810	21.4	15.6	1130	29.8	24.0
820	21.6	15.8	1140	30.2	24.4
830	22.0	16.2	1150	30.4	24.6
840	22.2	16.4	1160	30.6	24.8
850	22.4	16.6	1170	31.0	25.0
860	22.7	16.9	1180	31.3	25.5
870	22.9	17.1	1190	31.5	25.7
880	23.1	17.3	1200	31.7	25.9
890	23.5	17.7	1210	32.1	26.3
900	23.7	17.9	1220	32.3	26.5
910	24.0	18.2	1230	32.5	26.7
920	24.2	18.4	1240	33.0	27.2
930	24.6	18.8	1250	33.2	27.4
940	24.8	19.0	1260	33.4	27.6
950	25.0	19.2	1270	33.6	27.8
960	25.2	19.4	1280	34.1	28.3
970	25.5	19.7	1290	34.3	28.5
980	25.9	20.1	1300	34.5	28.7
990	26.1	20.3	1310	34.9	29.1
1000	26.3	20.5	1320	35.1	29.3
1010	26.5	20.7	1330	35.4	26.6
1020	26.9	21.1	1340	35.8	30.0
1030	27.2	21.4	1350	36.0	30.2
1040	27.4	21.6	1360	36.2	30.4
1050	27.6	21.8	1370	36.6	30.8
1060	28.0	22.2			

² Bieler und Schneidewind, Die agrikultur-chemische Versuchsstation, Halle, a/S., 1892 : 114.

³ Maercker and Delbrück, Handbook der Spiritus Fabrication, 1908 edition : 183.

Example.—Let the weight of a potato in air be 159 grams, and its weight in water 14.8 grams.

Then the weight of 10,000 grams of potatoes of like nature in water would be found from the equation $159:10,000 = 14.8:x$.

Whence $x = 931$ nearly.

In the table the nearest figure to 931 is 930, corresponding to 24.6 per cent. of dry matter and 18.8 per cent. of starch. When the number found is half way between the numbers given in the table the mean of the data above and below can be taken. In other positions a proper interpolation can be made if desired but for practical purposes the data corresponding to the nearest number can be used.

293. Constitution of Cellulose.—The group of bodies known as cellulose comprises many members of essentially the same chemical constitution but of varying properties. The centesimal composition of pure cellulose is shown by the following numbers:

Carbon,	44.2	per cent.
Hydrogen,	6.3	" "
Oxygen,	49.5	" "

corresponding to the formula $C_6H_{10}O_5$.

According to the view of Cross and Bevan, cellulose conforms in respect of its ultimate constitutional groups to the general features of the simple carbohydrates, but differs from them by reason of a special molecular configuration resulting in a suppression of the activity of constituent groups in certain respects, and an increase in activity of others.⁴

294. Fiber and Cellulose.—The carbohydrates of a plant insoluble in water are not composed exclusively of starch. There are, in addition to starch, pentosan bodies yielding pentose sugars on hydrolysis and furfuraldehyd on distillation with a strong acid. The quantitative methods for estimating the pentosan bodies have already been described.

In the estimation of cattle foods and of plant substances in gen-

⁴ Cross and Bevan, *Cellulose*, 2nd. Edition, 1903 : 77.

eral the residue insoluble in dilute boiling acid and alkali is called crude or indigestible fiber.

The principle on which the determination depends rests on the assumption that all the protein, starch and other digestible carbohydrates will be removed from the sample by successive digestion at a boiling temperature with acid and alkali solutions of a given strength. It is evident that the complex body obtained by the treatment outlined above is not in any sense a definite chemical compound, but it may be considered as being composed mostly of cellulose.

295. Official Method of Determining Crude Fiber.—The method of estimating crude fiber, adopted by the Association of Official Agricultural Chemists, is as follows:⁵

Extract two grams of the substance with ordinary ether, at least almost completely, or use the residue from the determination of the ether extract. To this residue, in a half liter flask, add 200 cubic centimeters of boiling 1.25 per cent. sulfuric acid; connect the flask with an inverted condenser, the tube of which passes only a short distance beyond the rubber stopper into the flask. Boil at once, and continue the boiling for 30 minutes. A blast of air conducted into the flask may serve to reduce the frothing of the liquid. Filter, wash thoroughly with boiling water until the washings are no longer acid, rinse the substance back into the same flask with 200 cubic centimeters of a boiling 1.25 per cent. solution of sodium hydroxid, free or nearly free of sodium carbonate, boil at once and continue the boiling for 30 minutes in the same manner as directed above for the treatment with acid. Filter into a gooch, and wash with boiling water until the washings are neutral, dry at 110°, weigh and incinerate completely. The loss of weight is crude fiber.

The filter used for the first filtration may be linen, one of the forms of glass wool or asbestos filters, or any other form that secures clear and reasonably rapid filtration. The solutions of sulfuric acid and sodium hydroxid are to be made up of the

⁵ Division of Chemistry, Bulletin 46, revised, 1899 : 26.

specified strength, determined accurately by titration and not merely from specific gravity.

The experience of many analysts has shown that results practically identical with those got as above, are obtained by conducting the digestions in hard glass beakers covered with watch glasses. The ease of manipulation in the modification of the process just mentioned is a sufficient justification for its use.

296. Solubility of Cellulose.—Cellulose resembles starch in its general insolubility, but, unlike starch, it may be dissolved in some reagents and afterwards precipitated practically unchanged or in a state of hydration. One of the simplest solvents of cellulose is zinc chlorid in concentrated aqueous solution.

The solution is accomplished with the aid of heat, adding one part by weight of cotton to six parts of zinc chlorid dissolved in 10 parts of water.

A homogeneous sirup is obtained by this process, which is used in the arts for making the carbon filaments of incandescent electric lamps, and other purposes.

In preparing the thread of cellulose, the solution, obtained as described above, is allowed to flow, in a fine stream, into alcohol, whereby a cellulose hydrate is precipitated, which is freed from zinc hydroxid by digesting in hydrochloric acid.

Hydrochloric acid may be substituted for water in preparing the reagent above noted, whereby a solvent is secured which acts upon cellulose readily in the cold.

A solution of ammoniacal cupric oxid is one of the best solvents for cellulose. The solution should contain from 10 to 15 per cent. of ammonia and from two to two and a half of cupric oxid.

In the preparation of this reagent, ammonium chlorid is added to a solution of cupric salt and then sodium hydroxid in just sufficient quantity to precipitate all of the copper as hydroxid. The precipitate is well washed on a linen filter, squeezed as dry as possible and dissolved in ammonia of 0.92 specific gravity. The cellulose is readily precipitated from the solution in cuprammonium by the addition of alcohol, sodium chlorid, sugar, or

other dehydrating agents. Solutions of cellulose are used in the arts for many purposes.⁶

297. Qualitative Reactions for Detecting Cellulose.—Cellulose may be identified by its resistance to the action of oxidizing agents, to the halogens and to alkaline solutions. It is further recognized by the sirupy or gelatinous solutions it forms with the solvents mentioned above. The cellulose hydrates precipitated from solutions have in some instances the property of forming a blue color with iodine.

A characteristic reaction of cellulose is secured as follows: To a saturated solution of zinc hydrochlorate, of 2.00 specific gravity, are added six parts by weight of potassium iodide dissolved in 10 parts of water and this solution is saturated with iodine. Cellulose treated with this reagent is at once stained a deep blue violet color.⁷ For the characteristics of cellulose occurring in wood the researches of Lindsey may be consulted.⁸

298. More Rarely Occurring Carbohydrates.—It is not possible here to give more space to the rarer forms of carbohydrates, to which the attention of the agricultural analyst may be called. Nearly 100 kinds of sugars alone have been detected in the plant world. For descriptions of the properties of these bodies and the methods of their detection and determination, the standard works on carbohydrates may be consulted.⁹

⁶ and ⁷ Bersch, *Cellulose*, 1904 : 8 et. seq. ; 15.

⁸ *Composition of Wood*, *Agricultural Science*, 1893, **7** : 49, 97 and 161.

⁹ Tollens, *Handbuch der Kohlenhydrate*, 1888, Von Lippmann, *Chemie der Zuckerarten*.

PART FOURTH.

FATS AND OILS.

299. Nomenclature.—The terms fat and oil are often used interchangeably and it is difficult in all cases to limit definitely their application. The consistence of the substance at usual room temperatures may be regarded as a point of demarcation. The term fat, in this sense, is applied to glycerids which are solid or semi solid, and oil to those which are quite or approximately liquid. A further classification is found in the origin of the glycerids, and this gives rise to the groups known as animal or vegetable fats and oils. In this manual, in harmony with the practices mentioned above, the term fat will be used to designate an animal or vegetable glycerid which is solid, and the term oil one which is liquid at common room temperature, *viz.*, about 20°. There are few animal oils, and few vegetable fats when judged by this standard, and it therefore happens that the term oil is almost synonymous with vegetable glycerid and fat with a glycerid of animal origin. Nearly related to the fats and oils is the group of bodies known as resins and waxes. This group of bodies, however, can be distinguished from the fats and oils by chemical characteristics. The waxes are ethers formed by the union of fatty acids and alcohols not belonging to the glycerol series.¹⁰ This chemical difference is not easily expressed and the terms themselves often add confusion to the meaning, as for instance, japan wax is composed mostly of fats, and sperin oil is essentially a wax.

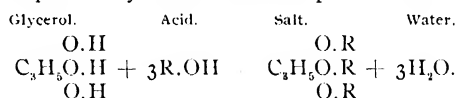
300. Composition.—Fats and oils are composed chiefly of salts produced by the combination of the complex base glycerol with the fat acids. Certain glycerids, as the lecithins, contain also phosphorus in organic combinations, nitrogen, and possibly other inorganic constituents in organic forms. By the action of alkalis

¹⁰ Lewkowitsch, *Chemical Technology and Analysis of Oils, Fats, Waxes*, 4th Edition, 1909 : Vol. 1 : pp. 2 and 48.

the glycerids are easily decomposed, the acid combining with the inorganic base and the glycerol becoming free. The salts thus produced from the soaps of commerce and the freed base, when collected and purified, is the glycerol of the trade.

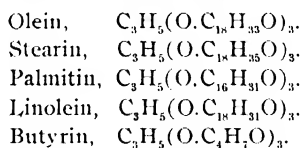
When waxes are decomposed by alkalies, fatty acids and alcohols of the ethane series are produced.

The natural glycerids formed from glycerol, which is a trihydric (triatomic) alcohol, are found in the neutral state composed of three molecules of the acid, united with one of the base. If R represent the radicle of the fat acid the general formula for the chemical process by which the salt is produced is:



The resulting salts are called triglycerids or neutral glyceryl ethers. In natural animal and vegetable products, only the neutral salts are found, the mono- and diglycerids resulting from artificial synthesis. For this reason the prefix tri is not necessarily used in designating the natural glycerids, stearin, for instance, meaning the same as tristearin.

301. Principal Glycerids.—The most important glycerids which the analyst will find are the following:



Olein is the chief constituent of most oils; palmitin is found in palm oil and many other natural glycerids; stearin is a leading constituent of the fats of beeves and sheep, and butyrin is a characteristic constituent of butter, which owes its flavor largely to this glycerid and its nearly related concomitants.

302. Extraction of Oils and Fats.—Preparatory to a physical and chemical study of the fats and oils is their separation from the other organic matters with which they may be associated. It

the case of animal tissues this is usually accomplished by the application of heat. The operation known as rendering may be conducted in many different ways. For laboratory purposes, the animal tissues holding the fat are placed in a convenient dish and a degree of heat applied which will liquify all the fat particles and free them from their investing membranes. The temperature employed should be as low as possible to secure the desired effect, but most fats can be subjected for some time to a heat of a little more than 100° , without danger of decomposition. The direct heat of a lamp, however, should not be applied, since it is difficult to avoid too high a temperature at the point of contact of the flame and dish. The dry heat of an air-bath or rendering in an autoclave or by steam is preferable. The residual animal matter is subjected to pressure and the combined liquid fat freed from foreign matters by filtering through paper in a steam or hot water funnel.

On a large scale, as in rendering lard, the fat is separated by steam in closed vats which are strong enough to withstand the steam pressure employed. For analytical purposes it is best to extract the fat from animal tissues in the manner described, since the action of solvents is slow on fat particles enveloped in their containing membranes, and the fats, when extracted, are liable to be contaminated with extraneous matters. In dried and ground flesh meal, however, the fat may be extracted with the usual solvents. For the quantitative determination of fat in bones or flesh, the sample, as finely divided as possible, is thoroughly dried, and the fat separated from an aliquot finely powdered portion by extraction with chloroform, ether, or petroleum. The action of anhydrous ether on dried and powdered animal matters is apparently a continuous one. Dormeyer has shown that even after an extraction of several months additional matter goes into solution.¹¹ The fat in such cases can be determined by saponification with alcoholic potash and the estimation of the free fatty acids produced.

¹¹ Archiv für Physiologie, 1895, 61 : 341 ; Chemiker-Zeitung Repertorium, 16 : 338.

From vegetable substances, such as seeds, the fat is extracted either by pressure or by the use of solvents. For quantitative purposes, only solvents are employed. The dry, finely ground material is exhausted with anhydrous ether or petroleum spirit, in one of the convenient forms of apparatus already described (48-56). In very oily seeds great difficulty is experienced in

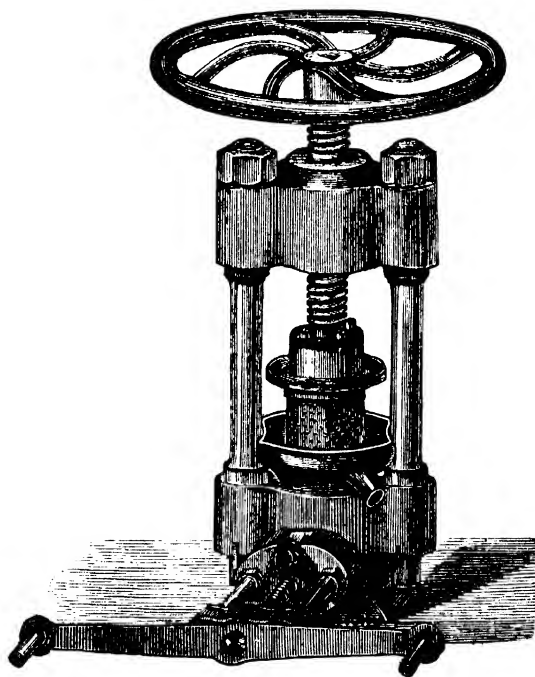


Fig. 74.—Oil Press.

securing a fine state of subdivision suited to complete extraction. In such cases it is advisable to conduct the process in two stages. In the first stage the material, in coarse powder, is exhausted as far as possible, either in a press or by means of a solvent and the percentage of oil determined. The residue is then

easily reduced to a fine powder, in an aliquot part of which the remaining oil is determined in the usual way.

In securing oils for physical and chemical examination both pressure and solution may be employed. The purest oils are secured by pressure at a low temperature. To obtain anything like a good separation some sort of hydraulic pressure must be used. An apparatus in which the first pressure is secured by a direct acting screw and this is supplemented by hydraulic pressure in which glycerol is the transmitting liquid gives good results. The construction of the press is shown in the accompanying figure.

The entire basket, filled with the pulverized material enclosed in a stout bag, and the collecting cup are warmed to 100° to 150° and then quickly put in the press and the large screw turned down as tight as possible. When the oil ceases to run out in a stream under this pressure the mass is further compressed by tightening up the horizontal screw shown at the bottom of the apparatus. This transmits its pressure very perfectly, through glycerin, to the plunger located beneath the catch saucer. A pressure of about 300 atmospheres is thus obtained. The material should be allowed to stand under pressure for several hours, the screw being tightened from time to time as the oil runs out.

Even with the best laboratory hydraulic pressure not nearly all the oil contained in oleaginous seeds can be secured and the process is totally inapplicable to securing the oil from tissues when it exists in quantities of less than 10 per cent. To get practically all of the oil the best method is to extract with carefully distilled petroleum of low boiling point.

In the preparation of this reagent the petroleum ether of commerce, (low boiling petroleum) containing bodies boiling at temperatures of from 35° to 80° , is repeatedly fractionated by distillation until a product is obtained which boils at from 45° to 70° . The purification of this material is conducted in a large flask heated with steam or upon an electric hot plate, the flask to be

connected with some convenient form of fractionating head, such as Glinsky's, and this with a condenser and adapter to the receiver, which should be packed in ice, as shown in Figure 76. When the crude gasoline is purchased in one gallon or smaller

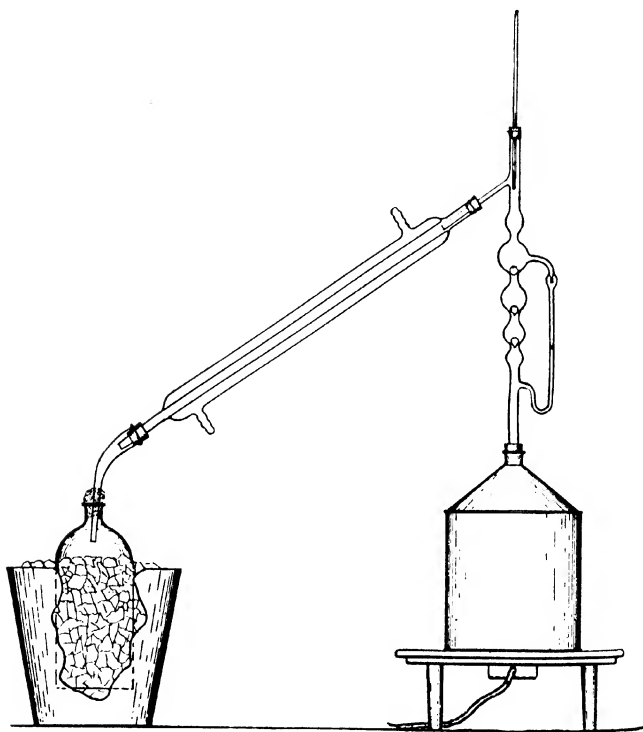


Fig. 75.—Fractionating Petroleum Ether.

tin containers having a smooth, round neck, it is not necessary to transfer to a glass flask as the tin can itself makes a very serviceable and safe distilling vessel. A thermometer suspended in the vapor of the petroleum serves to regulate the process. Too much

care in avoiding accidents can not be exercised in this operation. Not only must steam or electricity be used in heating, but all flames must be kept away from the vicinity of the apparatus, in fact it is advisable to carry on these fractionations in a room where there are no flames at all. In the beginning of the process, as much as possible of the petroleum boiling under 45° should be removed and rejected. The distillation is then continued until the temperature rises above 70° . The parts of the distillate saved between these temperatures are redistilled under similar conditions. Other portions of the petroleum, boiling at other temperatures, may be secured in the same way. The products may be in a measure freed of unpleasant odors by redistilling them from a mixture with lard. When used for quantitative purposes the petroleum ether must leave no residue upon evaporation.

303. Freeing Extracted Oils from Petroleum.—The petroleum ether which is used for extracting oils tends to give them an unpleasant odor and flavor and its entire separation is a matter of some difficulty. The greater part of the solvent may be recovered as described in paragraph 57. Heating the extracted oil for several hours in thin layers, will remove the last traces of the solvent, but affords opportunity for oxidation, especially in the case of drying oils. An effective means of driving off the last traces of petroleum is to pass a current of dry carbon dioxid through the sample contained in a cylinder and heated to a temperature of from 85° to 90° . The atmosphere of the inert gas will preserve the oil from oxidation and the sample will, as a rule, be found free of the petroleum odor after about 10 hours treatment. Ethyl ether or chloroform may be used instead of petroleum, but these solvents act on other matters than the glycerids, and the extract is therefore liable to be contaminated with more impurities than when the petroleum ether is employed. Other solvents for fats are carbon tetrachlorid, carbon disulfid, and benzene. Carbon tetra chlorid would be a most desirable solvent, owing to the fact that it is not inflammable and that it dissolves nearly all the fats and oils with ease, if it were not that the last traces of it are removed with the greatest difficulty and also that in the presence

of even minute traces of moisture it is partly decomposed into hydrochloric acid, which of course may act upon the fat and make it useless for further determinations. Its use therefore cannot be recommended where a pure extract is desired, but for some commercial operations it is meeting with favor now that the cost of its production has decreased. In general, petroleum ether should be employed in preference to other solvents, except in the case of castor oil, which is difficultly soluble in both petroleum and petroleum ethers.

304. Freeing Fats of Moisture.—Any excess of water in glycerids will accumulate at the bottom of the liquid sample and can be removed by decanting the fat or separating it from the oil by an other convenient method. The warm oil may be almost entirely freed of any residual moisture by passing it through a dry filter paper in a jacketed funnel kept at a high temperature. A section showing the construction of such a funnel with a folded filter paper in place, is shown in Fig. 76. The final drying, when great exactness is required, is accomplished in a vacuum oven or an atmosphere of some inert gas. In drying, it is well to expose the hot oil as little as possible to the action of the air.

305. Sampling for Analysis.—It is a matter of some difficulty to secure a representative sample of a fat or oil for analytical purposes. The moisture in a fat is apt to be unevenly distributed, and the sampling is to be accomplished in a manner to secure the greatest possible uniformity. Thus in sampling large quantities of solid products, a trier, which removes a cylinder through the entire depth of the material, should be used. One or more samples should be taken from each container and from a composite sample of each of these, a bulk sample made up, combining the smaller samples together in the same relative proportion represented by the individual containers to the whole shipment. The large sample should then be thoroughly mixed and quartered down to a suitable laboratory size. If practical, it is best to melt the entire bulk sample at a low temperature, and then stir con-

tinually as the mass cools in order to mix the water and impurities homogenously.¹²

In the case of butter fat the official chemists recommend that subsamples be drawn from all parts of the package until about 500 grams are secured. The portions thus drawn are to be per-

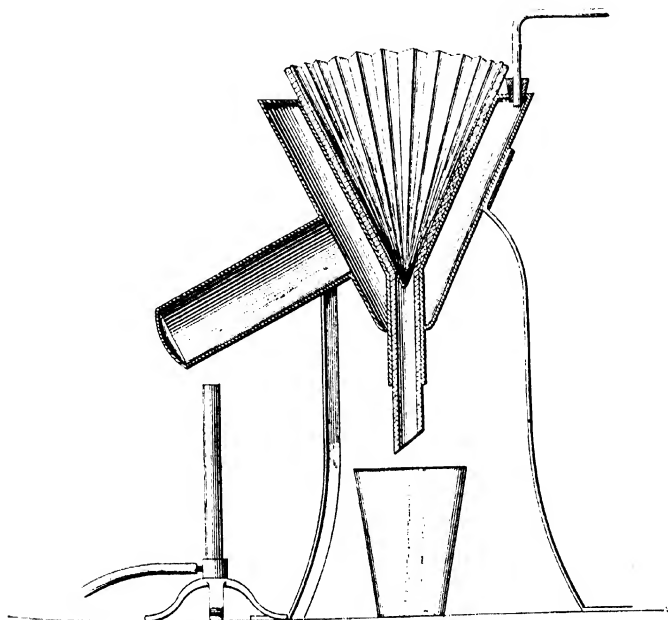


Fig. 76. Section Showing Construction of a Funnel for Hot Filtration.

fectly melted in a closed vessel at as low a temperature as possible, and when melted the whole is to be shaken violently for some minutes till the mass is homogenous, and sufficiently solidified to prevent the separation of the water and fat. A portion is then poured into the vessel from which it is to be weighed for analysis.

¹² Lewkowitsch, *Chem. Technology and Analysis of Oils, Fats, and Waxes*, 4th Edition, 1909, Vol. II, p. 209. See also Bulletin 107 (Rev.) 1908, Bureau of Chemistry, Department of Agriculture : 123.

and this should nearly or quite fill it. This sample should be kept in a cold place till analyzed.¹³

306. Estimation of Water.—In the official method for butter fat, which may be applied to all kinds, about two grams are dried to constant weight, at the temperature of boiling water, in a dish with flat bottom, having a surface of at least 20 square centimeters.

The use of clean dry sand or asbestos is admissible, and is necessary if a dish with round bottom be employed.

In the method recommended by Benedikt, about five grams of the sampled fat are placed in a small flask or beaker and dried at 100° with occasional stirring to bring the water to the surface.

According to the method of Sommenschein, the sample is placed in a flask carrying a cork, with an arrangement of glass tubes, whereby a current of dry air may be aspirated over the fat during the process of drying. When the flask is properly fitted its weight is taken, the fat put in and reweighed to get the exact amount. The fat is better preserved by aspirating carbon dioxide instead of air.¹⁴ The moisture may also be readily determined by drying on pumice stone, as described in the general directions for drying. In this case it is well to conduct the desiccation in vacuum or in an inert atmosphere to prevent oxidation.

PHYSICAL PROPERTIES OF FATS.

307. Specific Gravity.—The specific gravity of an oil is readily determined by a westphal balance, by a spindle, by a sprengel tube, or more accurately by a pycnometer. The general principles governing the conduct of the work have already been given. The methods described for determining the density of sugar solutions are essentially the same as those used for oils, but it is to be remembered that oils and fats are lighter than water and the graduation of the sinkers for the hydrostatic balance, and the spindles for direct determination must be for such lighter liquids. The necessity of determining the density of a fat at a tempera-

¹³ Division of Chemistry, Bulletin 46 revised, 1899 : 43.

Division of Chemistry, Bulletin 107 revised, 1912 : 129-147.

¹⁴ Journal of the Society of Chemical Industry, 1886, 5 : 508.

ture above its melting point is manifest, and for this reason the use of the pyknommeter at a high temperature (40° to 100°) is to be preferred to all the other processes, in the case of fats which are solid at temperatures below 25°.

In the determination of the specific gravity of solid fats such as lard and the still higher melting tallow, as well as the entire group of waxes the temperature of boiling water has been adopted. The method for making the determination officially adopted by the Association of Official Agricultural Chemists is as follows:¹⁵

(b) DETERMINATION AT 100° C.—OFFICIAL. — (1) *Standardization of Flasks.*—(a) *Method I.*—Use a small specific-gravity flask of from 25 to 30 cubic centimeters capacity. Wash the flask thoroughly with hot water, alcohol, and ether, and then dry it. After cooling in a desiccator accurately determine the weight of the flask and stopper. Fill the flask with freshly boiled, hot, distilled water. Keep the water of the bath in brisk ebullition for 30 minutes, any evaporation from the flask being replaced by the addition of boiling distilled water. Then insert the stopper, previously heated to 100°, remove the flask, wipe it dry, and after it has nearly cooled to room temperature place it in the balance and weigh when the balance temperature is reached.

(b) *Method II.*—The following formula may be used for calculating the weight of water (W^T) which a given flask will hold at T° (weighed in air with brass weights at the temperature of the room) from the weight of water (W^t) (weighed in air with brass weights at the temperature of the room) contained therein at t° :

$$W^T = W^t \frac{d^T}{d^t} [1 + y(T - t)]$$

d^T = the density of water at T° .

d^t = the density of water at t° .

y = the coefficient of cubical expansion of glass.¹⁶

¹⁵ Bureau of Chemistry, Bulletin 107 revised, 1908 : 130.

¹⁶ This factor is commonly given as 0.000026, but it varies considerably. Schultze, *Zeitschrift für analytische Chemie*, 1882, **21** : 167, found that the glass he used varied from 0.0000288 to 0.0000305 an average of 0.0000296. Division of Chemistry, Bulletin 62 : 121.

(2) DETERMINATION.—Rinse the flask with alcohol and ether and dry for a few minutes at the temperature of boiling water. Fill the flask with the dry, hot, fresh-filtered fat, which should be entirely free from air bubbles; replace it in the water bath, and keep for 30 minutes at the temperature of boiling water. Insert the stopper, previously heated to 100° C., remove the flask, wipe dry, place in the balance after it has nearly cooled to room temperature, and weigh when the balance temperature is reached. The weight of fat having been determined, obtain the specific gravity by dividing the weight of fat by the weight of water previously found. Example:

	Grams
Weight of flask, dry	16.0197
Weight of flask, plus water	37.3412
Weight of water	27.3215
Weight of flask, plus fat	34.6111
Weight of fat	24.5914

$$\text{Specific gravity} = 24.5914 \div 27.3215 = 0.90008.$$

The weight of the dry empty flask may be used constantly if great care be taken in handling and cleaning the apparatus, but the weight of water at boiling temperature must be determined under the barometric conditions prevailing at the time the determination is made.

When great delicacy of manipulation is desired, combined with rapid work, an analytical balance and westphal sinker may be used conjointly.¹⁷ In this case it is well to have two or three sinkers graduated for 20°, 25°, and 40°, respectively. Nearly all fats, when melted and cooled to 40°, remain in a liquid state long enough to determine their density. The sinkers are provided with delicate thermometers, and the temperature, which at the beginning is a little above the degree at which the sinker is graduated, is allowed to fall to just that degree, when the equilibrium is secured in the usual manner. The sinker is conveniently made to displace just five grams of distilled water at the temperature of graduation, but it is evident that a round number is not necessary, but only convenient for calculation.

¹⁷ Division of Chemistry, Bulletin 13, 1889 : 430.

308. Expression of Specific Gravity.—Much confusion arises in the study of data of densities because the temperatures at which the determinations are made are not expressed. The absolute specific gravity would be a comparison of the weight of the object at 4° , with water at the same temperature. It is evident that such determinations are not always convenient, and for this reason the determinations of density are usually made at other temperatures.

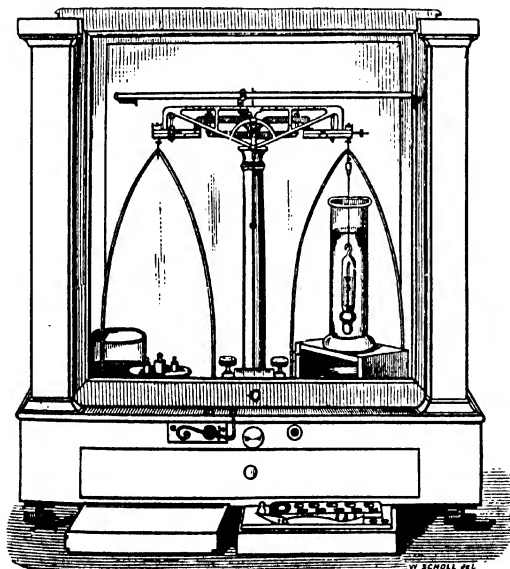


Fig. 77.—Balance and Westphal Sinker.

In the case of a sinker, which at 35° displaces exactly five grams of water, the following statements may be made: One cubic centimeter of water at 35° weighs 0.994098 gram. The volume of a sinker displacing five grams of water at that temperature is therefore 5.0297 cubic centimeters. This volume of water at 4° weighs 5.0297 grams. In a given case the sinker placed in an oil at 35° is found to displace a weight equal

to 4.5725 grams corresponding to a specific gravity of $\frac{345}{350} = 0.9145$. From the foregoing data the following tabular summary is constructed:

Weight of	5.0287	cubic centimeters of	oil at 35°	4.5725	grams.
"	"	5.0297	"	"	" water at 35°, 5.0000 "
"	"	5.0297	"	"	" " " 4°, 5.0297 "
Relative weight of oil at 35° to water at 35°.				0.9145	"
"	"	"	"	35°	" " 4°, 0.9092 "

309. Coefficient of Expansion of Oils.—Oils and fats of every kind have almost the same coefficient of expansion with increasing temperature. The coefficient of expansion is usually

calculated by the formula $\delta = \frac{D_0 - D_0'}{(t' - t)D_0}$ in which δ represents

the coefficient of expansion, D_0 the density at the lowest temperature, D_0' the density at the highest temperature, t the lowest, and t' the highest temperatures.

In the investigations made by Crampton it was shown that the formula would be more accurate, written as follows:¹⁸

$$\delta = \frac{D_0 - D_0'}{(t' - t) \sum_{t_0}^{t'} \frac{D_0}{D_0'}}$$

The absolute densities can be calculated from the formula $\Delta = \delta + K$, in which Δ represents the coefficient of absolute expansion, δ the apparent coefficient of expansions observed in glass vessels, and K the cubical coefficient of expansion of the glass vessel. The mean absolute coefficient of expansion for fats and oils, for 1° as determined by experiment, is almost exactly 0.0007, and the apparent coefficient of expansion nearly 0.00004.¹⁹

310. Standard of Comparison.—In expressing specific gravities it is advisable to refer them always to water at 4°. The temperature at which the observation is made should also be given.

¹⁸ Division of Chemistry, Bulletin 13, 1889 : 435.

¹⁹ Division of Chemistry, Bulletin 13, 1889 : 437.

Allen Commercial Organic Analysis, 3d Edition, 1899, 2 : 33.

Winton and Ogden, Connecticut Agricultural Experiment Station Report, 1900, Part 2 : 149.

Thus the expression of the specific gravity of lard, determined at different temperatures, is made as follows:

$$d_{\frac{15^{\circ}.5}{4^{\circ}}} = 0.91181; d_{\frac{40^{\circ}}{4^{\circ}}} = 0.89679; \text{ and } d_{\frac{100^{\circ}}{4^{\circ}}} = 0.85997,$$

indicating the relative weights of the sample under examination at $15^{\circ}.5$, 40° , and 100° , respectively, to water at 4° .

311. Densities of Common Fats and Oils.—It is convenient to have at hand some of the data representing the densities of common fats and oils, and the following numbers are from results of determinations made under my supervision:²⁰

Temperature.	$d_{\frac{15^{\circ}.5}{4^{\circ}}}$	$d_{\frac{40^{\circ}}{4^{\circ}}}$	$d_{\frac{100^{\circ}}{4^{\circ}}}$
Leaf lard	0.91181	0.89679	0.85997
Lard stearin	0.90965	0.89443	0.85750
Oleostearin	0.90714	0.89223	0.85572
Crude cottonseed oil	0.92016	0.90486	0.86739
Summer " "	0.92055	0.90496	0.86681
Winter " "	0.92179	0.90612	0.86774
Refined " "	0.92150	0.90573	0.86714
Compound lard "	0.91515	0.90000	0.86289
Olive oil	0.91505	0.89965	0.86168

312. Melting Point.—The temperature at which fats become sensibly liquid is a physical characteristic of some importance. Unfortunately, the line of demarcation between the solid and liquid states of this class of bodies is not very clear. Few of them pass *per saltum* from one state to the other. In most cases there is a gradual transition, which, between its initial and final points, may show a difference of several degrees in temperature. It has been noted, further, that fats recently melted behave differently from those which have been solid for several hours. For this reason it is advisable, in preparing glycerids for the determination of their melting point, to melt them the day before the examination is to be made. The temperature at which a glycerid passes from a liquid to a solid state is usually higher than that at which it resumes its solid form. If, however, the change of temperature could be made with extreme slowness, ex-

²⁰ Division of Chemistry, Bulletin 13, 1889 : 437.

posing the sample for many hours at near its critical temperature, these differences would be much less marked.

Many methods have been devised for determining the melting point of fats, and none has been found that is satisfactory in every respect. In some cases the moment at which fluidity occurs is assumed to be that one when the small sample loses its opalescence and becomes clear. In other cases the moment of fluidity is determined by the change of shape of the sample or by observing the common phenomena presented by a liquid body. In still other cases, the point at which the sample becomes fluid is determined by the automatic completion of an electric circuit, which is indicated by the ringing of a bell. This latter process has been found very misleading in our experience. Only a few of the proposed methods seem to demand attention here, and some of those, depending on the visible liquefaction of a small quantity of the fat or based on the physical property, possessed by all liquids when removed from external stress, of assuming a spheroidal state will be described. Other methods which may demand attention in any particular case may be found in the works cited.²¹ In stating melting point temperatures it is most important that the method used in the determination should be given and often desirable that both the temperature at which the solid begins to melt and that at which the sample becomes transparent should be noted.

313. Determination in a Capillary Tube.—A capillary tube is dipped into the melted fat and when filled one end of the tube is sealed in the lamp and it is then put aside in a cool place for 24 hours. At the end of this time the tube is tied to the bulb of a delicate thermometer the length used or filled with fat being of the same length as the thermometer bulb. The thermometer and attached fat are placed in water, oil, or other transparent media, and gently warmed until the capillary column of fat becomes transparent. At this moment the thermometer reading is made and entered as the melting point of the fat. In comparative

²¹ Lewkowitsch, *Chemical Technology and Analysis of Oils, Fats and Waxes*, 4th Edition, 1909 : 1 : 239-47.

Zune, *Analyse des Beurres*, 1892, 1 re partie : 26 et seq.

determinations the same length of time should be observed in heating, otherwise discordant results will be obtained. As in all other methods, the resulting figures are comparative and not absolute points of fusion, and the data secured by two observers on the same sample may not agree, if different methods of preparing the fat and different rates of fusion have been employed. A very convenient form of apparatus in which to heat the tube is shown in Fig. 79 under paragraph 314.

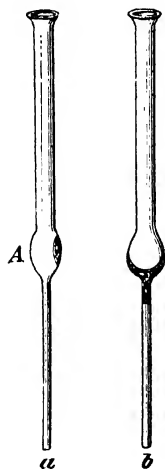


Fig. 78. - Melting Point Tubes.

Several modifications of the method just described are practiced, and perhaps with advantage in some cases. In one of these a small particle of the fat is solidified in a bulb blown on a small tube, as indicated in Fig. 78, tube *a*. The tube, in an upright position, is heated in a convenient bath until the particle of fat just begins to run assuming soon the position shown in tube *b*. This temperature is determined by a thermometer, whose bulb is kept in contact with the part of the observation tube containing the fat particle. The rise of temperature is continued until the

fat collected at the bottom of the bulb is entirely transparent. This is called the point of complete fusion.²²

Pohl covers the bulb of a thermometer with a thin film of fat, and the instrument is then fixed in a test tube, in such a way as not to touch the bottom, and the film of fat warmed by the air-bath until it fuses and collects in a droplet at the end of the thermometer bulb.²³

Carr has modified this process by inserting the thermometer in a round flask in such a way that the bulb of the thermometer is as nearly as possible in the center. By this device the heating through the intervening air is more regular and more readily controlled.²⁴

A particle of fat placed on the surface of clean mercury will melt when the mercury is raised to the proper temperature. Where larger quantities of the fat are employed, a small shot or pellet of mercury may be placed upon the surface and the whole warmed until the metal sinks. Of the above-noted methods, the analyst will find some form of capillary tube or the use of a film of the fat on the bulb of a thermometer the most satisfactory.²⁵

Hehner and Angell have modified the sinking point method by increasing the size of the sinker without a corresponding increase in weight. This is accomplished by blowing a small pear-shaped float, nearly one centimeter in diameter and about two long. The stem of the pear is drawn out and broken off, and while the bulb is still warm, the open end of the stem is held in mercury, and a small quantity of this substance, sufficient in amount to cause the float to sink slowly through a melted fat, is introduced into the bulb of the apparatus and the stem sealed. The whole bulb should displace about one cubic centimeter of liquid and weigh, after filling with mercury, between three and four-tenths grams. In conducting the experiment about 30

²² Journal of the Society of Chemical Industry, 1885, 4 : 535.

²³ Lewkowitsch, Chemical Technology and Analysis of Oils, Fats and Waxes, 4th Edition, 1909 : 1 : 239.

²⁴ Division of Chemistry, Bulletin 13, part 4, 1889 : 448.

²⁵ Lewkowitsch, Chemical Technology and Analysis of Oils, Fats and Waxes, 4th Edition, 1909 : 1 : 241.

grams of the dry melted fat are placed in a large test tube and cooled by immersing the tube in water at a temperature of 15° . The tube containing the solidified fat is placed in a bath of cold water and the sinker is placed in the center of the surface of the fat. The bath is slowly heated until the float disappears. The temperature of the bath is read just as the bulb part of the float disappears. The method is recommended especially by the authors for butter fat investigations.²⁶

314. Melting Point Determined by the Spheroidal State.—The method described by the author, depending on the assumption of the spheroidal state of a particle of liquid removed from all external stress, has been found quite satisfactory in this country and has been adopted by the official chemists.²⁷ In the preparation of the apparatus there are required:

(a) A piece of ice floating in distilled water that has been recently boiled, and (b) a mixture of alcohol and water of the same specific gravity as the fat to be examined. This is prepared by boiling distilled water and 95 per cent. alcohol for a few minutes to remove the gases which they may hold in solution. While still hot, the water is poured into the test tube described below until it is nearly half full. The test tube is then nearly filled with the hot alcohol, which is carefully poured down the side of the inclined tube to avoid too much mixing. If the alcohol is not added until the water has cooled, the mixture will contain so many air bubbles as to be unfit for use. These bubbles will gather on the disk of fat as the temperature rises and finally force it to the top.

A form of apparatus, which on account of its adaptability to both the spheroidal and capillary tube methods for the determination of melting points, is a convenient one to keep set up in the

²⁶ Hehner and Angell, *Butter, its Analysis and Adulterations*, 2nd Edition, 1877 : 24.

²⁷ Division of Chemistry, Bulletin 46 revised, 1899 : 52.

Division of Chemistry, Bulletin 107 revised, 1912 : 129.

laboratory is shown in Fig. 79. It consists of an accurate thermometer reading easily to tenths of a degree, suspended in a large test tube 30 centimeters long and three and a half in

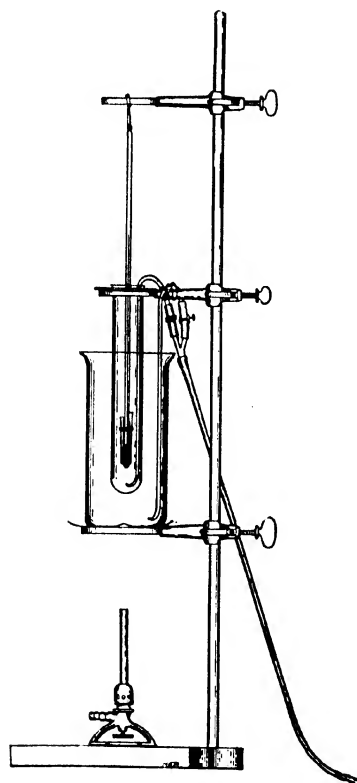


Fig. 79.—Apparatus for the Determination of Melting Point.

diameter. This latter is supported in the center of a tall beaker about 35 centimeters high and 10 in diameter, resting on an asbestos gauze over any suitable burner. Two tubes drawn down

and bent at their lower ends are fixed, one in each vessel and attached through a Y to the blast, (a rubber blowing bulb or small foot bellows may be used if no power blast is convenient). On each of the rubber tubes that connect the glass stirrers to the Y a screw pinch cock is placed to facilitate the regulation of the air. In using this apparatus with the spheroidal method the blast must of course be shut off entirely from the inner tube. For very exact work a cathetometer may be used to read the thermometer but as a usual thing it is less difficult to make the reading than to determine the exact time when it should be made.

The apparatus may also with advantage have an asbestos plaque inserted between the lamp and the tubes to more carefully control the temperature.

The disks of fat are prepared as follows: The melted and filtered fat is allowed to fall from a dropping tube from a height of about 20 cubic centimeters on a smooth piece of ice floating in recently boiled distilled water. The disks thus formed are from one to one and a half centimeters in diameter and weigh about 200 milligrams. By pressing the ice under the water the disks are made to float on the surface, whence they are easily removed with a steel spatula, which should be cooled in the ice water before using. They should be prepared a day or at least a few hours before using.

Steenbock²⁸ proposes to substitute for the cake of ice a cold mercury surface and it has been found that if the melted fat, held at a carefully controlled temperature, be dropped from the proper height disks of a very uniform size and weight can be made in this way. As soon as cool the disks should be removed from the mercury with a cold spatula and dropped into a beaker of 50 per cent. alcohol and the beaker then placed in a desiccator under vacuum until all the small air bubbles which at first collect upon the disks have disappeared.

The test tube containing the alcohol and water is placed in a tall beaker, containing water and ice, until cold. The disk of

²⁸ *Journal of Industrial and Engineering Chemistry*, **2** (1910) 480.

fat is then dropped into the tube from the spatula, and at once sinks until it reaches a part of the tube where the density of the alcohol-water is exactly equivalent to its own. Here it remains at rest and free from the action of any force save that inherent in its own molecules.

The delicate thermometer is placed in the test tube and lowered until the bulb is just above the disk. In order to secure an even temperature in all parts of the alcohol mixture in the vicinity of the disk, the thermometer is gently moved from time to time in a circle.

The disk having been placed in position, the water in the beaker is slowly heated, and kept constantly stirred by means of the blowing apparatus already described.

When the temperature of the alcohol-water mixture rises to about 6° below the melting point, the disk of fat begins to shrivel, and gradually rolls up into an irregular mass.

The thermometer is now lowered until the fat particle is even with the center of the bulb. The bulb of the thermometer should be small, so as to indicate only the temperature of the mixture near the fat. A gentle rotatory movement from time to time should be given to the thermometer bulb. The rise in temperature should be so regulated that the last 2° of increment require about 10 minutes. The mass of fat gradually approaches the form of a sphere, and when it has gathered into a globule the reading of the thermometer is to be made. As soon as the temperature is taken the test tube is removed from the bath and placed again in the cooler. A second tube, containing alcohol and water, is at once placed in the bath. The test tube (ice water having been used as a cooler) is of low enough temperature to cool the bath sufficiently. After the first determination, which should be only a trial, the temperature of the bath should be so regulated as to reach a maximum of about $1^{\circ}.5$ above the melting point of the fat under examination.

The edge of the disk should not be allowed to touch the sides

of the tube. This accident rarely happens, but in case it should take place, and the disk adhere to the sides of the tube, a new trial should be made.

Triplicate determinations should be made, and the second and third results should show a near agreement.

Example.—Melting point of sample of butter:

	Degrees.
First trial.....	33.15
Second trial.....	33.05
Third trial.....	33.00

The fatty acids, being soluble in alcohol, cannot be treated as the ordinary glycerids. But even those glycerids which are slightly soluble in alcohol may be subjected to the above treatment without fear of experiencing any grave disturbance of the fusing points.

315. Solidifying Point.—The temperature at which a fat shows incipient solidification is usually lower than the point of fusion. The same difficulties are encountered in determining the temperature of solidification as are presented in observing the true melting point. The passage from a transparent liquid to an opaque solid is gradual, showing all the phases of turbidity from beginning opalescence to complete opacity. The best the analyst can do is to determine, as accurately as possible, the temperature at which the more solid glycerids of the mixture begin to form definite crystals. This point is affected to a marked degree by the element of time. A fat cooled just below its melting point will become solid after hours, or days, whereas it could be quickly cooled far below that temperature and still be limpid.

The methods of observation are similar for the glycerids and fatty acids, and the general process of determination is sufficiently set forth in the following description.²⁹

316. Titer Test.—In the commercial valuation of soap stock and other fats, the solidifying point of the fatty acids known to the trade as the Titer Test is now almost universally applied, and inasmuch as the method of preparing these acids affect materially

²⁹ Division of Chemistry, Bulletin 13, part 4, 1889 : 447.

the final results, the process recommended by the A. O. A. C. is here given in full.

(a) STANDARD THERMOMETER.—The thermometer must be graduated in tenth degrees from 10° to 60° , with a zero mark, and have an auxiliary reservoir at the upper end, also one between the zero mark and the 10° mark. The cavity in the capillary tube between the zero mark and the 10° mark must be at least one centimeter below the 10° mark, the 10° mark to be about three or four centimeters above the bulb, the length of the thermometer being about 35 centimeters. The thermometer is annealed for 75 hours at 450° , and the bulb is of Jena normal 16¹¹¹ glass, moderately thin, so that the thermometer will be quick acting. The bulb is about three centimeters long and six millimeters in diameter. The stem of the thermometer is six millimeters in diameter and made of the best thermometer tubing, with scale etched on the stem, the graduation to be clear cut and distinct, but quite fine.

(b) DETERMINATION.—Saponify 75 grams of fat in a metal dish with 60 cubic centimeters of 30 per cent. sodium hydroxid (36° Baumé) and 75 cubic centimeters of 95 per cent. by volume alcohol or 120 cubic centimeters of water. Boil to dryness, with constant stirring to prevent scorching, over a very low flame or over an iron or asbestos plate. Dissolve the dry soap in a liter of boiling water, and if alcohol has been used boil for 40 minutes in order to remove it, adding sufficient water to replace that lost in boiling. Add 100 cubic centimeters of 30 per cent. sulfuric acid (25° Baumé) to free the fatty acids, and boil until they form a clear, transparent layer. Wash with boiling water until free from sulfuric acid, collect in a small beaker, and place on the steam bath until the water has settled and the fatty acids are clear; then decant them into a dry beaker, filter using hot-water funnel, and dry 20 minutes at 100° . When dried, cool the fatty acids to 15° or 20° above the expected titer and transfer to the titer tube, which is 25 millimeters in diameter and 100 millimeters in length and made of glass about one millimeter in thickness. Place in a half liter saltmouth bottle of clear glass, about 70 milli-

meters in diameter and 150 millimeters high, fitted with a cork, which is perforated so as to hold the tube rigidly when in position. Suspend the thermometer, graduated to 0.10° , so that it can be used as a stirrer, and stir the mass slowly until the mercury remains stationary for 30 seconds. Then allow the thermometer to hang quietly, with the bulb in the center of the mass, and observe the rise of the mercury. The highest point to which it rises is recorded as the titer of the fatty acids.

Test the fatty acids for complete saponification as follows:

Place three cubic centimeters in a test tube and add 15 cubic centimeters of alcohol (95 per cent. by volume). Bring the mixture to a boil and add an equal volume of ammonium hydroxid (0.96 sp. gr.). A clear solution should result, turbidity indicating unsaponified fat. The titer must be made at about 20° for all fats having a titer above 30° and at 10° below the titer for all other fats.³⁰

317. Determination of Refractive Power.—The property of refracting light is possessed by fats in different degrees and these differences are of great help in analytical work. The examination may be made by the simple refractometer of Abbe or Bertrand, the more elaborate apparatus of Pulfrich or by the immersion refractometer.³¹

The comparative refractive power of fats can also be observed by means of the oleorefractometer of Amagat-Jean or the differential refractometer of Zune.³²

For details of the construction of these apparatus, with a description of the optical principles on which they are based, the papers above cited may be consulted. In my investigations the instruments which have been employed are three in number, *viz.*, Abbe's small refractometer, Pulfrich's refractometer using yellow

³⁰ Bureau of Chemistry, Circular 22.

³¹ Pulfrich, *Das totalreflectometer und das Refractometer für Chemiker*, 1894; Abbe, *neu apparate zur Bestimmung des Brechungs-und Zerstreuungsvermögens fester und flüssiger Körper*. Jena. 1874.

³² Zune, *Analyse des Beurres*, 2 e partie, 1892 : 33 et 63; *Zeitschrift für Instrumentenkunde*, 1887, : 16, 55, 392, 444; *Zeitschrift für physikalische Chemi*, 18 : 294.

light, and the oleorefractometer of Amagat-Jean. A brief description of the methods of manipulating these instruments is all that can be attempted in this manual.

318. Refractive Index.—Refractive index is an expression employed to characterize the measurement of the degree of deflection caused in a ray of light in passing from one transparent medium into another. It is the quotient of the sine of the angle of the incident, divided by the sine of the angle of the refracted ray.

In the case of oils which remain liquid at room temperatures, the determinations can be made without the aid of any device to maintain liquidity. In the case of fat which becomes solid at ordinary room temperatures, the determination must either be made in a room artificially warmed or the apparatus must have some device, as in the later instruments of Abbe and Pulfrich and in the apparatus of Amagat-Jean, whereby the sample under examination can be maintained in a transparent condition. In each case the accuracy of the apparatus should be tested by pure water, the refractive index of which at 18° is 1.333. The refractive index is either read directly on the scale as in Abbe's instrument, or calculated from the angles measured as in Pulfrich's apparatus.

319. Abbe's Refractometer.—For practical use the Abbe refractometer fitted with temperature controlled prisms shown in Fig. 80 is in common use. Water from any suitable constant temperature bath is circulated around the prisms entering at D and leaving through E. A thermometer with its bulb enclosed in the water jacket near the water outlet serves to indicate their exact temperature. To charge the instrument the telescope prisms and all are rotated on the bearing (*a*), the eye piece (*e*) resting on the table, away from the observer, and the small lock screw (*v*) turned and the prism B thrown back leaving A exposed. In working with very fluid substances it is desirable to have the surface of A nearly horizontal and this may be done by

moving L, which is attached to the prisms only until the surface is level, (o) still resting on the bench thus leaving both hands free to apply the sample. This should be done with a small dropper or pipette, care being taken that the tube does not touch the glass of the instrument as there is danger of scratching its highly polished surface. One or two drops suffice and they need not be evenly distributed as when B is closed again and locked in place they are squeezed out into a very thin even layer. The

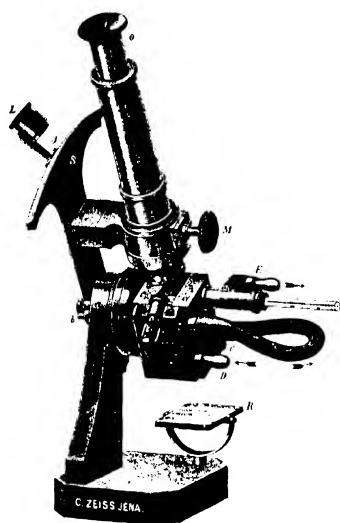


Fig. 80. Abbe's Refractometer with Heatable Prisms.

apparatus is placed in its normal position and the index moved until the light directed through the apparatus by the mirror shows the field of vision divided into dark and light portions. The dispersion apparatus 'T' is now turned by means of the screw M, until the rainbow colors on the part between the dark and light fields have disappeared. Before doing this, however, the telescope, the eyepiece of the apparatus, is so adjusted as to bring

the cross lines of the field of vision distinctly into focus. The reading glass *L* of the apparatus is now moved back and forth until the line of the two fields of vision falls exactly at the intersection of the cross lines. The refractive index of the fat under examination is then read directly upon the scale by means of this small magnifying glass. To check the accuracy of the first reading, the dispersion apparatus should be turned through an angle of 180° until the colors have again disappeared, and, after adjustment, the scale of the instrument again read. These two readings should nearly coincide, and their mean should be taken as the true reading of the fat under examination.

It is sometimes very difficult to work with this instrument in warm, humid, weather, at low temperatures because of the rapid deposit of moisture on the outer surfaces of the prisms which are not easily wiped off owing to their protected position. To obviate this difficulty we have found it very convenient to direct blasts of dry air previously passed over calcium chlorid against these surfaces by means of a couple of small tubes.

For butter fats the apparatus should be kept in a warm place, the temperature of which does not fall below 30° . For reducing the results obtained to a standard temperature, say 25° , for most oils the factor 0.000365 per degree centigrade calculated by Tolman and Munson³³ or 0.00038 given by Richmond³⁴ may be used but as this factor varies in different parts of the scale and for different oils the readings should be made at the standard temperature. For butter the correction is about 0.000176 per degree.

Example.—Refractive index of a butter fat determined at $32^\circ.4 = 1.4540$, reduced to 25° as follows: $32.4 - 25 = 7.4$; $0.000176 \times 7.4 = 0.0013$; then $1.4540 + 0.0013 = 1.4553$.

In the determination above given, the refractive index of pure water measured 1.3300; hence the above numbers should be corrected for theory by the addition of 0.0030, making the corrected

³³ Journal of the American Chemical Society, 1902, **24** : 755.

³⁴ The Analyst, 1907, **32** : 46.

index of the butter fat mentioned at the temperature given, 1.4583.

320. Pulfrich's Refractometer.—For exact scientific measurements, Pulfrich's apparatus has given entire satisfaction. In this instrument a larger quantity of the oil is required than for the Abbe, and this quantity is held in a cylindrical glass vessel luted to the top of the prism. The method of accomplishing this and also an illustration of the refraction of the rays of light are shown in Fig. 81.

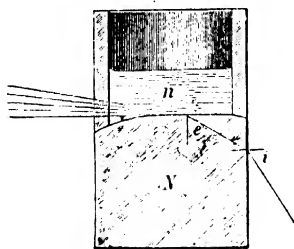


Fig. 81.—Prism of Pulfrich's Refractometer.

The angle i is measured by a divided circle read with the aid of a small telescope. The index of the prism of highly refractive glass N is known. The oil is seen at n . The light used is the yellow sodium ray (D). From the observed angle the refractive index of n is calculated from the formula $n = \sqrt{N^2 - \sin^2 i}$.

For convenience the values of n for all usual values of i are computed once for all and arranged for use in tabular form. The latest model of Pulfrich's apparatus, arranged both for liquid and solid bodies, and also for spectrometric observation is shown in Fig. 82.

When the sodium light is used it is placed behind the apparatus and the light is collected and reflected on the refractive prism

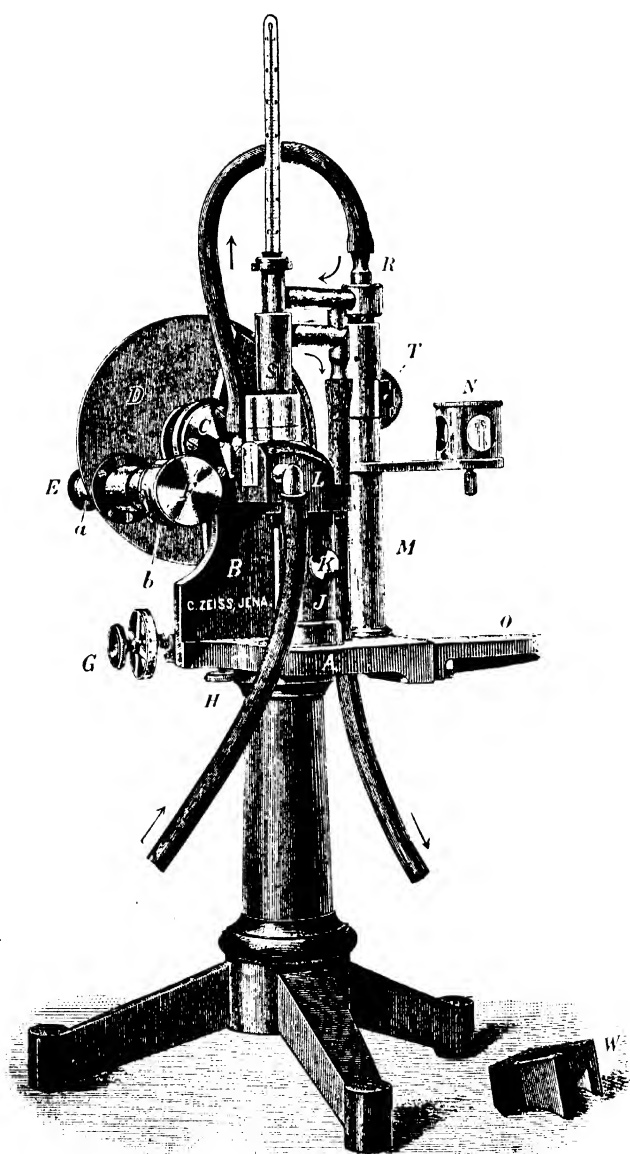


Fig. 82.—Pulfrich's New Refractometer.

by the lens *N*. Through *H* and *G* is secured the micrometric reading of the angle on the scale *D* by means of the telescopic arrangement *F E*. For regulating the temperature of the oil and adjacent parts, a stream of water at any desired temperature is made to circulate through *I* and *S* in the direction indicated by the arrows. The manner in which this is accomplished is shown in the cross section of that part of the apparatus as indicated in Fig. 83.

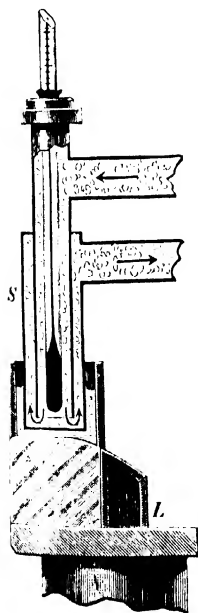


Fig. 83.—Heating Apparatus for Pultrich's Refractometer.

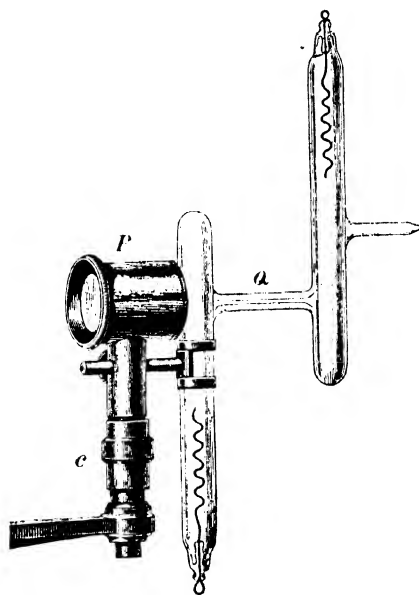


Fig. 84.—Spectrometer Attachment.

For further details of the construction and operation of the apparatus the original description may be consulted.³⁵

In case a spectrometric observation is desired the *H* ray, for

instance, is produced by the Geissler tube *Q*, Fig. 84. The light is concentrated and thrown upon the refractive prism by the lens *P*, the lens *N*, Fig. 83, being removed for this purpose.

Tables, for correcting the dispersion and for calculating the indices for each angle and fraction thereof, and for corrections peculiar to the apparatus, accompany each instrument.

321. Refractive Indices of Some Common Oils.—The following numbers show the refractive indices obtained by Long for some of the more common oils. The light used was the yellow ray of the sodium flame.³⁶

Name.	Temperature.	Refractive index.	Calculated for 38°.
Olive oil (France).....	26° 6	1.4673	1.4677
" " (California).....	25° 4	1.4677	1.4678
Cottonseed oil	24° 8	1.4722	1.4721
" "	26° 3	1.4703	1.4709
" "	25° 3	1.4718	1.4719
Sesamé oil.....	24° 8	1.4728	1.4728
" "	26° 8	1.4710	1.4716
Castor "	25° 4	1.4771	1.4773
Lard "	27° 3	1.4657	1.4666
Peanut "	25° 3	1.4696	1.4696

In case of the use of Abbe's apparatus, in which diffused sunlight is the source of the illumination, the numbers obtained cannot be compared directly with those just given unless the apparatus be first so adjusted as to read with distilled water at 18°, 1.333. In this case the reading of the scale gives the index as determined by the yellow ray. The numbers obtained with Abbe's instrument for some common oils are given below.³⁷

In the determinations the instrument was set with water at 18°, reading 1.3300, and they were corrected by adding 0.0030 in order to compensate for the error of the apparatus.

Material.	Calculated for 25°.	Corrected index.
Lard.....	1.4620	1.4650
Cotton oil	1.4674	1.4704
Olive oil stearin	1.4582	1.4610
Lard stearin.....	1.4594	1.4624

³⁶ American Chemical Journal, 1888, 10 : 392.

³⁷ Division of Chemistry, Bulletin 13, part 4, 1889 : 473 et. seq.

322. Oleorefractometer.—Instead of measuring the angular value of the refractive power of an oil it may be compared with some standard on a purely arbitrary scale. Such an apparatus is illustrated by the oleorefractometer of Amagat-Jean, or by Zeiss's butyrorefractometer.

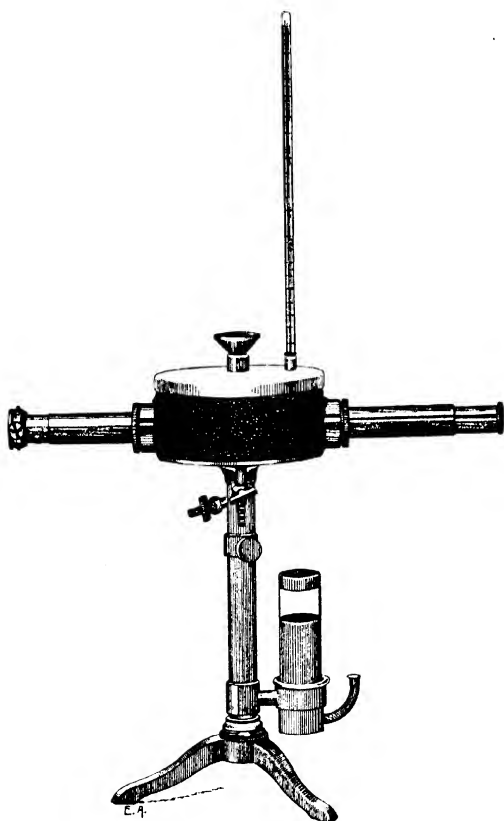


Fig. 85. —Oleorefractometer.

In the first named instrument, Fig. 85, the oil to be examined is compared directly with another typical oil and the shadow pro-

duced by the difference in refraction is located on a scale read by a telescope and graduated for two different temperatures.³⁸ The internal structure of the apparatus is shown in Fig. 86.

In the center of the apparatus a metal cylinder, *A*, is found carrying two plate glass pieces, *C B*, so placed as to form an angle of 107° . This cylinder is placed in a larger one, provided with two circular glass windows. To these two openings are fixed to the right and left, the telescopic attachments, *G, F, S, E*, and the apparatus *M, H, S', E'*, for rendering the rays of light

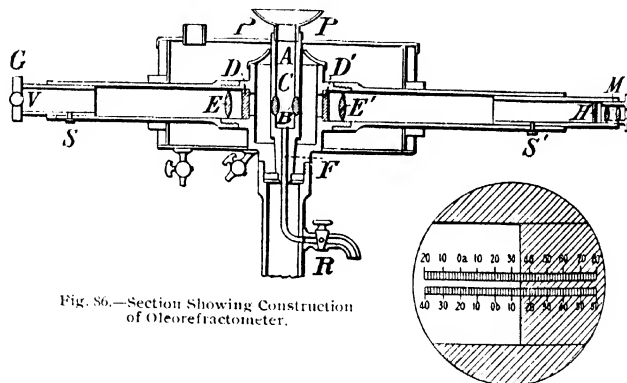


Fig. 86.—Section Showing Construction of Oleorefractometer.

parallel. The field of vision is divided into two portions, light and dark, by a semicircular stop inserted in the collimator, and contains the double scale shown in the figure placed at *H*. The field of vision is illuminated by a gas or oil lamp placed at a convenient distance from the collimator. The inner metallic cylinder *A* is surrounded with an outer one, to which the optical parts are attached at *D D'* by means of plane glass plates. This cylinder is in turn contained in the large water cylinder *P P*, carrying a thermometer in the opening shown at the top on the left. The manipulation of the apparatus is very simple. The outer cylinder is filled with water, at a temperature below 22° , the middle one with the typical oil furnished with the instrument, the

³⁸ Jean, *Chimie Analytique des Matières Grasses*, 1892 : 26.

cover of the apparatus carrying the thermometer placed in position and the cup-shaped funnel inserted in the cylinder *A*, which is at first also filled with the typical oil. The whole system is next brought slowly to the temperature of 22° by means of the lamp shown in Fig. 92. The telescope is adjusted to bring the scale of the field of vision into focus and the line dividing the light and shadow of the field should fall exactly on $o^{\circ}a$. If this be not the case the o° is adjusted by screws provided for that purpose until it is in proper position. The typical oil is withdrawn from *A* by the cock *R*, the cylinder washed with a little of the oil to be examined and then filled therewith. On again observing the field of vision the line separating the shadow from the light will be found moved to the right or left, if the oil have an index different from that of the typical oil. The position of the dividing line is read on the scale.

For fats the temperature of the apparatus is brought exactly to 45° and the scale $o^{\circ}b$ is used. In other respects the manipulation for the fats is exactly that described for oils. In the use of $o^{\circ}a$, in case the room be warmer than 22° , all the liquids employed should be cooled below 22° before being placed in the apparatus. It is then only necessary to wait until the room temperature warms the system to 22° . In the case of fats it is advisable to heat all the liquids to about 50° and allow them to cool to 45° instead of heating them to that temperature by means of the lamp.

According to Jean³⁹ the common oils, when purified, give the following readings at 22° :

Peanut oil.....	+ 3.5 to + 6.5
Colza ".....	+ 17.5 " + 21.0
Cotton ".....	+ 18.0 " + 18.0
Linseed ".....	+ 47.0 " + 54.5
Lard ".....	+ 5.5 " + 5.5
Olive ".....	+ 1.5 " 0.0
Sesamé ".....	+ 17.5 " + 19.0
Oleomargarin.....	- 15.0 " - 15.0
Butter fat.....	- 30.0 " - 30.0
Mutton oil.....	0.0 " 0.0
Fish ".....	+ 38.0 " + 38.0

³⁹Jean, *Chimie Analytique des Matières Grasses*, 1892 : 31 et seq.

In this instrument, therefore, vegetable and fish oils, as a rule, show a right hand, and animal fats a left hand deviation.

We have not found, however, the values fixed by Jean to be constant. The numbers for lard have varied from -3.0 to -10.0 , and other fats have shown almost as wide a variation from the values assigned by him.

Jean states that the number for lard, determined by the oleo-refractometer, is -12 , and he gives a definite number for each of the common oils and fats. On trying the pure lards of known origin in this instrument, I have never yet found one that showed a deviation of -12 divisions of the scale; but I have no doubt that there are many such lards in existence. The pure normal lards derived from the fat of a single animal, would naturally show greater variations in their chemical and physical properties, than a typical lard derived from the mixed fats of a great many animals. In leaf lard, rendered in the laboratory, the reading of the oleorefractometer was found to be -10° , while with the intestinal lard it was -9° . On the other hand, a lard rendered from the fat from the back of the animal showed a reading of only -3° , and a typical cottonseed oil a reading of $+12^{\circ}$. According to the statement of Jean, a lard which gives even as low a refractive number as -9 , by his instrument, would be adjudged at least one-quarter cottonseed oil.

After a thorough trial of the instrument of Jean, I am convinced that it is of great diagnostic value, but if used in the arbitrary manner indicated by the author it would lead to endless error and confusion. In other words, this instrument is of greater value in analyses than Abbe's ordinary refractometer, because it gives a wider expansion in the limits of the field of vision, and therefore can be more accurately read, but it is far from affording a certain means of discovering traces of adulteration with other fats.

323. Variations in the Instruments.—In the use of the oleo-refractometer, attention should be called to the fact that, through some negligence in manufacture, the instruments do not give, in all instances, the same reading with the same substance. Allen

obtained the following data with a sample of lard examined in three instruments, *viz.*, 4°.5, 6°, and 11°. Such wide differences in the scales of the instruments cannot fail to disparage the value of comparative determinations.

The variations in samples of known origin, when read on the same instrument, however, will show the range of error to which the determinations made with the oleorefractometer are subject. Pearmain has tabulated a large number of observations of this kind, covering 240 samples of oils.⁴⁰

Following are the data relating to the most important oils.

AT 22°			
Name of oil.	Highest reading. Degrees.	Lowest reading. Degrees.	Mean reading. Degrees.
Almond	10.5	8.0	9.5
Peanut	7.0	5.0	6.0
Castor	42.0	39.0	40.0
Codliver	46.0	40.0	44.0
Cottonseed (crude)	17.0	16.0	16.5
" (refined)	23.0	17.0	21.5
Lard oil	1.0	0.0	0.0
Linseed (crude)	52.0	48.0	50.0
" (refined)	54.0	50.0	52.5
Olive	3.5	1.0	2.0
Rape	20.0	16.0	17.5
Sesamé	17.0	13.0	15.5
Sunflower	35.0	35.0	35.0
Tallow oil	5.0	1.0	3.0
Oleic acid	33.0	29.0	32.0
AT 45°.			
Butter	34.0	25.0	30.0
Oleomargarin	18.0	13.0	15.0
Lard	14.0	8.0	10.5
Tallow	18.0	15.0	16.0
Paraffin	58.5	54.0	56.0

324. Butyrorefractometer.—Another instrument graduated on an arbitrary scale is the butyrorefractometer of Zeiss. This apparatus, which resembles in some respects the instrument of Abbe, differs therefrom essentially in dispensing with the revolving prisms of Amici, whereby the chromatic fringing due to

⁴⁰ The Analyst, 1895, 20 : 135.

dispersion is corrected, and on having the scale fixed for one substance, in this instance, pure butter fat. The form of the instrument is shown in Fig. 87. The achromatization for the butter fat is secured in the prisms between which a film of the fat is placed, as in the Abbe instrument. When a fat, differing from that for which the instrument is graduated is introduced, the fringes of the dark and light portions of the field will not only be colored (difference in dispersion), but the line of separation

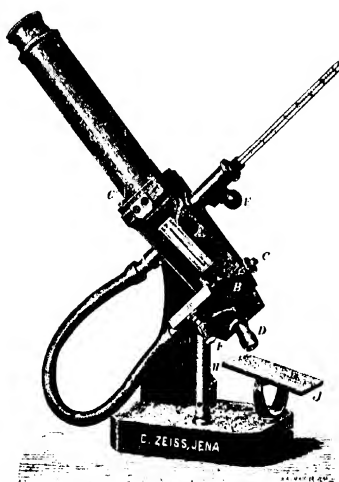


Fig. 87.—Butyrorefractometer.

will also be displaced (difference in refractive power). The apparatus is therefore used in the differential determination of these two properties. It must not be forgotten, however, that butter fats differ so much in these properties among themselves as to make possible the condemnation of a pure as an adulterated sample.

325. Method of Charging the Apparatus.—The prism casing of the instrument is opened by turning the pin *F* to the right and pushing the half *B* of the prism casing aside. The prism and its appendages must be cleaned with the greatest care, the best

means for this purpose being soft clean linen moistened with a little alcohol or ether.

Melt the sample of butter in a spoon and pour it upon a small paper filter held between the fingers and apply the first two or three drops of clear butter fat so obtained to the surface of the prism contained in prism casing *B*. For this purpose the apparatus should be raised with the left hand so as to place the prism surface in a horizontal position.

Press *B* against *A* and replace *F* by turning it in the opposite direction into its original position; thereby *B* is prevented from falling back and both prism surfaces are kept in close contact.

326. Method of Observation.—While looking into the telescope, give the mirror *J* such a position as to render the critical line which separates the bright left part of the field from the dark right part distinctly visible. It may also be necessary to move or turn the instrument about a little. First it will be necessary to ascertain whether the space between the prism surfaces be uniformly filled with butter, for, if not, the critical line will not be distinct.

By allowing a current of water of constant temperature to flow through the apparatus, some time previous to the taking of the reading, the somewhat hazy critical line approaches in a short time, generally after a minute, a fixed position and quickly attains its greatest distinctness. When this point has been reached note the appearance of the critical line (*i. e.*, whether colorless or colored and in the latter case of what color); also note the position of the critical line on the centesimal scale, which admits of the tenth divisions being conveniently estimated, and at the same time reading the thermometer. By making an extended series of successive readings and by employing an assistant for melting and preparing the small samples of butter, from 25 to 30 refractometric butter tests may, after a little practice, be made in an hour.

The readings of the refractive indices of a large number of butter samples made at 25° are, by means of a table which will

be found below, directly reduced to scale divisions and yield the following equivalents.⁴¹

Natural butter	(1.4590—1.4620)	: 49.5—54.0 scale divisions.
Margarin	(1.4650—1.4700)	: 58.6—66.4 " "
Mixtures	(1.4620—1.4690)	: 54.0—64.8 " "

Whenever, in the refractometric examination of butter at a temperature of 25°, higher values than 54.0 are found for the critical lines these samples will, according to Wollny, by chemical analysis, always be found to be adulterated; but in all samples in which the value for the position of the critical line does not fall below 52.5, chemical analysis may be dispensed with and the samples may be pronounced to be pure butter.

In calculating the position of the critical line for other temperatures than 25° allow for 1° variation of temperature a mean value of 0.55 scale division. The following table, which has been compiled in this manner, shows the values corresponding to various temperatures, each value being the upper limit of scale divisions admissible in pure butter:

Temp.	Sc. div.	Temp.	Sc. div.	Temp.	Sc. div.	Temp.	Sc. div.
45°	41.5	40°	44.2	35°	47.0	30°	49.8
44°	42.0	39°	44.8	34°	47.5	29°	50.3
43°	42.6	38°	45.3	33°	48.1	28°	50.8
42°	43.1	37°	45.9	32°	48.6	27°	51.4
41°	43.7	36°	46.4	31°	49.2	26°	51.9
40°	44.2	35°	47.0	30°	49.8	25°	52.5

If, therefore, at any temperature between 45° and 25° values be found for the critical line, which are less than the values corresponding to the same temperature according to the table, the sample of butter may safely be pronounced to be natural, i. e., unadulterated butter. If the reading show higher numbers for the critical line the sample should be reserved for chemical analysis. A special thermometer for use in the examination of butter will be described in the section devoted to dairy products.

The process of observation is precisely the same as that already described. In cases, however, where the critical line presents

⁴¹ Schlussbericht über die Butteruntersuchungsfrage, Milchwirthschaftlicher Verein Korrespondenzblatt, 39, 1891 : 15.

very broad fringes (turpentine, linseed oil, etc.) it is advisable to repeat the reading with the aid of a sodium flame.

327. Range of Application of the Butyrorefractometer.—The extended range of the ocular scale of the refractometer, $n = 1.42$ to 1.49 , which embraces the refractive indices of the majority of oils and fats, renders the instrument applicable for testing oils and fats and also for examining glycerol.

By reference to the subjoined table the scale divisions may be transformed into terms of refractive indices.

The following table is used to convert the degrees of the instrument into refractive indexes:

BUTYRO-REFRACTOMETER READINGS AND CORRESPONDING
INDEXES OF REFRACTION.⁴²

Reading	Index of refraction	Reading	Index of refraction	Reading	Index of refraction	Reading	Index of refraction
40.0	1.4524	50.0	1.4593	60.0	1.4659	70.0	1.4723
40.5	1.4527	50.5	1.4596	60.5	1.4662	70.5	1.4726
41.0	1.4531	51.0	1.4600	61.0	1.4665	71.0	1.4729
41.5	1.4534	51.5	1.4603	61.5	1.4668	71.5	1.4732
42.0	1.4538	52.0	1.4607	62.0	1.4672	72.0	1.4735
42.5	1.4541	52.5	1.4610	62.5	1.4675	72.5	1.4738
43.0	1.4545	53.0	1.4613	63.0	1.4678	73.0	1.4741
43.5	1.4548	53.5	1.4616	63.5	1.4681	73.5	1.4744
44.0	1.4552	54.0	1.4619	64.0	1.4685	74.0	1.4747
44.5	1.4555	54.5	1.4623	64.5	1.4688	74.5	1.4750
45.0	1.4558	55.0	1.4626	65.0	1.4691	75.0	1.4753
45.5	1.4562	55.5	1.4629	65.5	1.4694	75.5	1.4756
46.0	1.4565	56.0	1.4633	66.0	1.4697	76.0	1.4759
46.5	1.4569	56.5	1.4636	66.5	1.4700	76.5	1.4762
47.0	1.4572	57.0	1.4639	67.0	1.4704	77.0	1.4765
47.5	1.4576	57.5	1.4642	67.5	1.4707	77.5	1.4768
48.0	1.4579	58.0	1.4646	68.0	1.4710	78.0	1.4771
48.5	1.4583	58.5	1.4649	68.5	1.4713	78.5	1.4774
49.0	1.4586	59.0	1.4652	69.0	1.4717	79.0	1.4777
49.5	1.4590	59.5	1.4656	69.5	1.4720	79.5	1.4780

328. Polarization.—The pure glycerids are generally neutral to polarized light. In oils the degree of polarization obtained is often variable, sometimes to the right and sometimes to the left. Olive oil, as a rule, shows a slight right hand polarization.

⁴² Winton, Connecticut Agriculture Experiment Station Report, 1900-Part 2 : 143.

Peanut, sesame, and cottonseed oils vary in polarizing power, but in no case is it very marked. Castor oil polarizes slightly to the right.

The rotation appears to be due to two distinct causes. In the more common vegetable and animal oils, where it is very slight, it is doubtless dependent upon the non-glyceridic substances such as cholesterol and phytosterol, but Lewkowitsch⁴³ has shown that oils of the chaulmoogra group, which have rotations as high as $+57^{\circ}$ in a 200 millimeter tube, contain optically active fatty acids. The rotation of castor oil, $+6.4^{\circ}$, is doubtless due to ricinoleic acid which contains an asymmetric carbon atom.

In determining polarizing power of an oil it should be obtained in a perfectly limped state by filtration and observed through a tube of convenient length, as a rule, 200 millimeters. The deviation may be expressed in divisions of the sugar scale of the instrument or in degrees of angular rotation. The gyrodynic of the common oils has no important influence as an analytical factor.⁴⁴

329. Viscosity.—An important property of an oil, especially when its lubricating qualities are considered, is the measure of the friction which the particles exert on other bodies and among themselves, in other words, its viscosity. In the measure of this property no definite element can be considered, but the analyst must be content with comparing the given sample with the properties of some other liquid regarded as a standard. The usual method of procedure consists in determining the time required for equal volumes of the two liquids to pass through an orifice of given dimensions, under identical conditions of temperature and pressure. In many instances the viscosity of oils is determined by comparing them with water or rape oil, while, in other cases, a solution of sugar is employed as the standard of measurement.

In England the viscosimeter devised by Redwood,⁴⁵ which depends upon the time necessary for a certain quantity of oil to

⁴³ Chemical Technology and Analysis of Oils, Fats and Waxes, 4th Edition, 1909, 1 263.

⁴⁴ E. Louise and E. Sauvage. *Comptes rendus* 1907, Vol. 2 : 145, 188.

⁴⁵ *Journal of Society of Chemical Industry*, 1886, 5 : 126.

flow through a standard orifice at a standard temperature, is used. A somewhat similar piece of apparatus which, however, handles a much larger volume of oil than the Redwood instrument, was devised by Engler⁴⁶ and has been adopted by the German government as the standard instrument for use in that country. The apparatus of Saybolt,⁴⁷ which, however, can not be purchased as its manufacture is controlled by the Standard Oil Company, is used by this corporation in the valuation of all of their oil products. Tagliabue has, gotten out a slightly modified form of this instrument and in this country his apparatus has found considerable favor among oil manufacturers.

In case rape oil be taken as a standard and its viscosity represented by 100 the number representing the viscosity of any other oil may be found by multiplying the number of seconds required for the outflow of 50 cubic centimeters by 100 and dividing by 535. If the specific gravity vary from that of rape oil, *viz.*, 0.915, at 15°, a correction must be made by multiplying the result obtained above by the specific gravity of the sample and dividing the product by 0.915. If n be the observed time of outflow in seconds and s the specific gravity the viscosity is expressed as follows:⁴⁸

$$V = \frac{n \times 100 \times s}{535 \times 0.915} = \frac{n \times 100 \times s}{489.525}$$

It is important that the height of the oil in the cylinders from which it is delivered be kept constant, and this is secured by supplying additional quantities, on the principle of the mariotte bottle.

330. The Torsion Viscosimeter.—The torsion viscosimeter, based on the principle described by Babcock is frequently used. The instrument employed is the one described by Doolittle.⁴⁹ The construction of the apparatus is illustrated in Fig. 88.

⁴⁶ Journal of Society of Chemical Industry, 1893, 12, 291.

Zeitschrift für angewandte Chemie, 1892, 725-27.

⁴⁷ Redwood, Petroleum, 2nd Edition, 1906, 2 : 604.

⁴⁸ Lewkowitch, Chemical Technology and Analysis of Oils, Fats and Waxes, 4th Edition, 1909, 1 268.

⁴⁹ Journal of the American Chemical Society, 1893, 15 : 173.

A steel wire is suspended from a firm support and fastened to a stem which passes through a graduated horizontal disk, thus permitting the accurate measurement of the torsion of the wire. The disk is adjusted so that the index point reads exactly 0, thus showing that there is no torsion in the wire. A brass cylinder seven centimeters long by five in diameter, having a slender stem

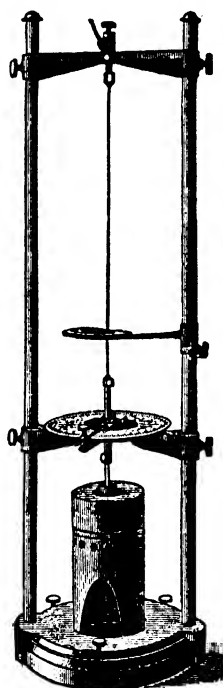


Fig. 88.—Doolittle's Viscosimeter.

by which to suspend it, is immersed in the oil and fastened by a thumbscrew to the lower part of the stem of the disk. The oil cup is surrounded by a bath of water or high fire-test oil, according to the temperature at which it is desired to determine the viscosity. This temperature obtained, while the disk is resting on its supports, the wire is twisted 360° by rotating the milled

head at the top. The disk being released, the cylinder rotates in the oil by virtue of the torsion of the wire.

The action now observed is identical with that of the simple pendulum.

If there were no resistance to be overcome, the disk would return to 0, and the momentum thus acquired would carry it 360° in the opposite direction. But the resistance of the oil to the rotation of the cylinder causes the revolution to fall short of 360° , and the greater the viscosity of the oil the greater will be the resistance, and also the retardation. This retardation is found to be a very delicate measure of the viscosity of the oil.

This retardation may be read in a number of ways, but the simplest is to read directly the number of degrees of retardation between the first and second complete arcs covered by the rotating pendulum. For example, suppose the wire be twisted 360° and the disk released so that rotation begins. In order to obtain an absolute reading to start from, which shall be independent of any slight error in adjustment, ignore the starting point and make the first reading of the index at the end of the first swing. The disk is allowed to complete a vibration and the needle is read again at its nearest approach to the first point read. The difference in the two readings will measure the retardation due to the viscosity of the liquid. In order to eliminate errors duplicate determinations are made, the milled head being rotated in an opposite direction in the second one. The mean of the two readings will represent the true retardation. Each instrument is standardized in a solution of pure cane sugar, as proposed by Babcock, and the viscosity, in each case, is a number representing the number of grams of sugar in 100 cubic centimeters, which, at 22° , would produce the retardation noted.

Each instrument is accompanied by a table which contains the necessary corrections for it and the number expressing the viscosity corresponding to the different degrees of retardation, as read on the index. The following numbers, representing the

viscosity of some oils as determined by the method of Doolittle, were obtained by Krug.⁶⁰

Peanut oil	48.50
Olive "	53.00
Cottonseed oil	46.25
Linseed "	33.50

331. Microscopic Appearance.—When fats are allowed to slowly crystallize from an ethereal solution they may afford crystalline forms, which, when examined under a microscope, yield valuable indications of the nature and origin of the substance under examination.⁶¹

The method of securing fat crystals for microscopic examination is as follows: From two to five grains of the fat are placed in a test tube and dissolved in 10 cubic centimeters of ether. The tube is loosely stoppered with cotton and allowed to stand, for 15 hours or longer, in a moderately cool room where no sudden changes of temperature are likely to take place. The most characteristic crystals are obtained when the crystallization proceeds slowly and at a temperature of 22° to 24° C. The first crop of crystals may be examined and the mother liquor separated and set aside for further crystallization.⁶² It is advisable to prepare several solutions of the same substance with varying proportions of solvent, for it is not possible to secure in a given instance those conditions which produce the most characteristic crystals. The rate and time of the crystallization should be such that the microscopic examination can take place when only a small portion of the fat has separated in a crystalline condition. A drop of the mass containing the crystals is removed by means of a pipette, placed on a slide, a drop of cotton or olive oil added, a cover glass gently pressed down on the mixture and the preparation subjected to microscopic examination. Several slides should be prepared from the same or different crystallizations. Sometimes the results of an examination made in this way are very definite, but the analyst must be warned not to expect definite data in

⁶⁰ Communicated by Krug to author.

⁶¹ Division of Chemistry, Bulletin 13, part 4, 1889 : 449 et seq.

⁶² Bureau of Chemistry, Bulletin 107, revised, 1908 : 147.

all cases. Often the microscopic investigations result in the production of negative or misleading observations, and, at best, this method of procedure must be regarded only as helpful and confirmatory.

A modification of the method of preparation described above has been suggested by Gladding.⁵³ About five grams of the melted fat are placed in a small Erlenmeyer dissolved in a mixture of 10 cubic centimeters of absolute alcohol mixed with half the quantity of ether. The flask is stoppered with a plug of cotton and allowed to stand in a cool place for about half an hour. By this treatment the more easily crystallizable portions of the fat separate in a crystalline form, while the triolein and its nearly related glycerids remain in solution. The crystalline product is separated by filtration through paper wet with alcohol and washed once with the solvent mentioned above. After drying in the air for some time the crystals are removed from the paper and dissolved in 25 cubic centimeters of ether, the cotton plug inserted, and the Erlenmeyer placed in a standing position, in a large beaker containing water. The water jacket prevents any sudden changes of temperature and affords an opportunity for the uniform evaporation of the ether which should continue for 15 hours or longer in a cool place.

Other solvents, viz., alcohol, chloroform, carbon disulfid, carbon tetrachlorid, petroleum and petroleum ether have been extensively used in the preparation of fat crystals for microscopic examination, but in our experience none of these is equal to ether when used as already described.

332. Distinctive Appearance of Crystals of Fats.—In the case of lard, there is a tendency, more or less pronounced, to form prismatic crystals with rhombic ends. Beef fat on the other hand shows a tendency to form fan-shaped crystals in which the radii are often curved.

Typical crystals of swine and beef fat are shown in the accompanying figures, 89 and 90.⁵⁴ In mixtures of swine and beef

⁵³ Journal of the American Chemical Society, 1896, 18 : 189.

⁵⁴ Division of Chemistry, Bulletin 13, part 4, 1889 ; Plates 32 and 35.



Fig. 89.—Lard Crystals X 65.



Fig. 90.—Compound Lard (beef fat) Crystals X 65.

fats the typical crystals are not always developed, but in most cases the fan-shaped crystals of the beef fat will appear more or less modified when that fat forms 20 per cent. or more of the mixture. When only five or 10 per cent. of the beef fat on the one hand or a like amount of swine fat on the other are present the expectation of developing any characteristic crystals of the minimum constituent is not likely to be realized.

The typical crystals of lard are thought by some experts to be palmitin and those of beef fat stearin, but no direct evidence has been adduced in support of these *a priori* theories.

Crampton,⁵⁵ has shown that the differences between the typical crystallization of beef and swine fats are plain. In mixed fats, on the contrary, confusing observations are often made. In a mixture of 10 per cent. of beef and 90 per cent. of swine fats a uniform kind of crystallization is observed, not distinctly typical, but the characteristics of the lard crystals predominate. In many cases a positive identification of the crystals is only made possible by repeated crystallizations. In the examination of so-called refined lards, which are mixtures of lard and beef fat, the form of aggregation of the crystals is found to resemble the fan-shaped typical forms of beef fat. When the single crystals, however, are examined with a higher magnifying power, they are not found to be pointed but blunt, and some present the appearance of plates with oblique terminations, but not so characteristic as those obtained from pure lard. In other cases in compound lards no beef fat crystals are observed and these lards may have been made partly of cotton oil stearin. When a lard crystal presents its edge to observation it may readily escape identification, or may even be mistaken for a crystal of beef fat. In order to insure a side view the cover glass should be pressed down with a slight rotatory movement, whereby some of the lard crystals at least may be made to present a side view.

333. Observation of Fat Crystals with Polarized Light.—The appearance of fat crystals, when observed by means of polarized

⁵⁵ Division of Chemistry, Bulletin 13, Part 1, 1887: 452, 29 et seq.

light alone or with the adjunct of a selenite plate, is often of value in distinguishing the nature and origin of the sample.⁵⁶

Every fat and oil which is amorphous will present the same set of phenomena when observed with polarized light through a selenite plate, but when a fat has been melted and allowed to cool slowly the field of vision will appear mottled and particolored when thus examined. This method has been largely used in the technical examination of butter for adulterants, and the microscope is extensively employed by the chemists of the Bureau of Internal Revenue for this purpose. In the examination of the crystals of butter fat by polarized light a cross is usually observed when the nicols are turned at the proper angle, but the cross, while almost uniformly seen with butter, is not distinctive, since other fats often show it. These forms of crystals are best obtained by heating the butter fat to the boiling-point of water for about a minute and then allowing it to slowly solidify, and stand for 24 hours.

Pure butter, properly made, is never subjected to fusion, and hence, when examined through a selenite plate, presents a uniform field of vision similarly illuminated and tinted throughout. In oleomargarin, the fats are sometime, during their preparation, in a fused condition. The field of vision is therefore filled to a greater or less extent with crystals more or less perfect in form. Some of these crystals, being doubly refracting, will impart to a selenite field a mottled appearance. Such a phenomenon in a supposed butter, is therefore indicative of a fraudulent addition or of one which has been at some time subjected to a temperature at or above its fusing point.

334. Spectroscopic Examination of Oils.—The presence of chlorophyll or of its alteration products is a characteristic of crude oils of vegetable origin. In refined oils, even when of a vegetable origin, all traces of the chlorophyll products may disappear. The absorption bands given by oils are not all alike and in doubtful cases a suspected sample should be compared with one of known origin.

⁵⁶ Division of Chemistry, Bulletin 13, Part 1, 1887 : 452, 29 et seq.

In conducting the examination, the oil in a glass vessel with parallel sides, is placed in front of the slit of the spectroscope and any absorption band is located by means of the common divided scale and by the color of the spectrum on which it falls. Olive and linseed oils give three sharply defined absorption bands, a very dark one in the red, a faint one on the orange and a well marked one in the green.

Sesame, arachis, poppyseed and cottonseed oils also show absorption bands. Castor and almond oils do not affect the spectrum.

Rape and flaxseed oils absorb a part of the spectrum but do not affect the rest of it. The spectroscope is at present of little practical utility in oil analysis.⁵⁷

335. Critical Temperature of Dissolution.—The study of the critical temperature of dissolution of oils has been made by Crismer, who finds it of value in analytical work.⁵⁸ If a fatty substance be heated under pressure, with a solvent, *e. g.*, alcohol, it will be noticed that as the temperature rises the meniscus of separation of the two liquids tends to become a horizontal plane. If at this point the contents of the tube be thoroughly mixed by shaking and then be left at rest, a point will soon be reached at which the two liquids again separate, and this point is distinctly a function of temperature. Following is a description of a convenient method of determining the critical temperature of the solution of fats and oils for experimental purposes. Tubes are prepared for holding the reagents in such a way that, after the introduction of the fat and alcohol, they can be easily sealed. The capacity of these tubes should be about five cubic centimeters.

⁵⁷ Vogel, *Practische Spectralanalyse*, 1877 : 279 ; Zunc, *Analyse des Beures* 1892, 2 : 48.

⁵⁸ Lewkowitsch, *Chemical Technology and Analysis of Oils, Fats and Waxes*, 4th Edition, 1909 : Vol. 1, 265.

Bulletin d l'Association Belge des Chimistes, 1895, 9 : 71, 145 ; 1896, 9 : 320 ; 1896, 10 : 312 ; *Liege Congrès* 1905, Sec. 1 : 323.

They should be charged with about one cubic centimeter of the dry filtered fat and about twice that quantity of 95 per cent. alcohol. Care should be exercised to avoid touching the upper sides of the tube with the reagents. When charged the tubes are sealed in the flame of a lamp and attached to the bulb of a delicate thermometer in such a manner as to have the surface of its liquid contents even with the top of the bulb. The tube is conveniently fastened to the thermometer by a platinum wire. For duplicate determinations two tubes may be fastened to the same thermometric bulb. The apparatus thus prepared is placed in a large vessel filled with strong sulfuric acid. The operator should be careful to protect himself from the danger which might arise from an explosion of the sealed tubes during heating. It is advisable in all cases to observe the reaction through a large pane of clear glass. The bath of sulfuric acid is heated by any convenient means and an even temperature throughout the mass is secured by stirring with the thermometer and its attachments. When the meniscus which separates the two liquids becomes a horizontal plane the thermometer is removed and the liquid in the tubes well mixed until it appears homogeneous. The thermometer is replaced in the bath, which is allowed to cool slowly, and the phenomena which take place in the sealed tubes are carefully noted. The critical temperature of dissolution is that at which the two liquids begin to separate. This moment is easily noted. It is, moreover, preceded by a similar phenomenon taking place in the capillary part of the tube which retains a drop of the mixture on shaking. In this droplet an opalescence is first noted. In the mass of the liquid this opalescence, a few seconds afterwards, is observed to permeate the whole, followed by the formation of zones and finally of the reappearance of the meniscus of separation between the two liquids. The temperature at this moment of opalescence preceding the separation of the liquid is the critical temperature of dissolution. With alcohol of 0.8195 specific gravity, at 15°.5 (95 per cent.), the observed critical temperatures for some of the more common fats and oils are as given below:

Butter fat.....	98°-103°
Oleomargarin.....	122°-126°
Peanut oil.....	115°-123°
Cotton ".....	116°
Olive ".....	123°
Sesamé ".....	121°
Colza ".....	132°-135°
Mutton tallow.....	116°
Beef marrow.....	125°
Nut oil.....	100°

When the alcohol is not pure or if it be of a different density from that named, the numbers expressing the critical temperature of solution will vary from those given above. Asbóth⁵⁹ has also determined the critical points for 90 per cent. alcohol.

336. Turbidity Temperature.—The turbidity temperature of a fat, when dissolved in glacial acetic acid, as suggested by Valenta, may prove of some diagnostic value. The fats are dissolved, with the aid of heat, in glacial acetic acid and, on slowly cooling, the temperature at which they become turbid is observed. It is important in this test that the acetic acid be absolutely glacial. About three cubic centimeters of the glacial acetic acid, and three of the fat, should be used.

Valenta⁶⁰ used equal volumes of acetic acid of 1.0562 specific gravity and the sample, and on the basis of the turbidity temperature divided the more common oils into three groups as follows: First, completely soluble at ordinary temperatures (*i. e.* below 23°), castor oil; second, oils completely soluble between 23° and the boiling point of the acetic acid solution, pumpkin seed, cotton seed, sesame, olive, peanut, almond, apricot kernel, cod liver, palm, nutmeg butter, cocoa butter, cocoanut, palm nut oils, and bone and butter fat, beef tallow and stearin; third group includes all oils insoluble at boiling point of the acid such as the rape oils, mustard, etc. In view of the discordant figures reported by Allen⁶¹ and Hoton⁶² little value can be placed in exact

⁵⁹ Chemiker Zeitung 1896 : 685.

⁶⁰ Allen, Journal of the Society of Chemical Industry, 1884, 3 : 643.

⁶¹ Allen, Journal of the Society of Chemical Industry, 1886, 5 : 69, 282.

⁶² Hoton, Bulletin d l'Association Belge des Chimistes, 1904, 2.

clouding temperatures, but the test is useful as a preliminary one and especially as a means of detecting adulteration of castor oil.

337. Solubility.—With the exception of castor oil, all the natural glycerides are soluble in petroleum ether and very slightly soluble in alcohol at ordinary temperatures. All oils and fats are freely soluble in ether, chloroform, carbon bisulfid, acetone, carbon tetrachlorid, and some less commonly used solvents, but the difference between the solubility of the various oils and different samples of the same oil is so slight and at the same time values given in the literature are so discordant, that such methods as Horsley's⁶³ for differentiating butter fat and lard and Milliau's⁶⁴ for detecting adulterants in olive oil must be considered of little use except as confirmatory of other tests.

As a general rule, oils rich in the lower fatty acids, as palm nut, cocoanut, porpoise, butter fat, etc., are more soluble in absolute alcohol than those composed chiefly of olein, palmitin and stearin. The solubility is very materially affected by the presence of free fatty acid, so much so that in the presence of 50 per cent. thereof solution is complete.

CHEMICAL PROPERTIES.

338. Importance of Chemical Study.—It has been shown in the preceding pages that the physical characteristics of fats and oils are of the utmost importance from a practical standpoint, as determining the particular economic use for which a particular oil is suited, and are often very valuable aids to the analysis of unknown samples, or detection of adulteration. But it is necessary for the chemist to go farther and determine what are known

⁶³ Chemical News, 1861, 230.

⁶⁴ Rapport sur les Procédés, à employer, pour reconnaître les Falsifications des Huiles d'Olive, 1895 : 37 ; *Annales de la Science Agronomique*, 1894-1895 ; 2 : 206.

Journal of the Chemical Society, Abstracts, 1884, 46 : 1078 ; Dingier's *Polytechnisches Journal*, 1884, 252 : 296.

The Analyst, 1894, 19 : 152.

Müntz, Durand, Milliau.

as the chemical constants. The natural oils and fats, as has been said, are complex mixtures of the glycerids of different fatty acids and were it possible to resolve them into their separate, component radicals quantitatively, the question of their analysis would be comparatively simple. Our present knowledge of the exact composition of the natural glycerids and the method at our disposal for their examination are such that we must reluctantly turn from a systematic chemical analyses of this class of substances to the more or less empirical determination of what are called by Hübl "quantitative reactions." In addition to these, many color and thermal reactions and special qualitative indices, such as the claidin, bromin and iodine tests, have been proposed and are more or less characteristic for special oils.

339. Coloration Produced by Oxidants.—When oils and fats are mixed with oxidizing reagents, such as sulfuric and nitric acids, the glycerids are partly decomposed with the production of colors which have some analytical significance. The most simple method of applying these tests is by the use of a thick porcelain plate provided with small cup-shaped depressions for holding the few drops of material required. Two or three drops of the oil under examination are placed in each of the cups, a like quantity of the oxidizing reagent added, and the mixture stirred with a small glass rod. The colors produced are carefully noted and the mixture is allowed to remain at room temperature for at least 12 hours in order that the final tint may be observed. The sulfuric acid used for this reaction should have a specific gravity of one and seven-tenths and the nitric acid should have the usual commercial strength of the strongest acid. Pure lard, when treated with sulfuric acid, as above described, shows but little change of color while the vegetable oils mostly turn brown or black. In addition to the reagents mentioned many others, including sulfuric and nitric acids, sulfuric acid and potassium bichromate, chlorine, ammonia, hydrogen peroxid, sodium hydroxid and aqua regia are used. Only a few of these tests seem to have sufficient analytical importance to merit any detailed description.

340. Coloration in Large Masses.—Instead of applying the color test in the small way just described, larger quantities of the fat may be used, either in the natural state or after solution in petroleum or other solvent. For this purpose about 10 cubic centimeters of the oil are shaken with a few drops of sulfuric acid or sulfuric and nitric acids. Lard, when thus treated (five drops of sulfuric acid to 10 cubic centimeters of lard) shows practically no coloration. When treated with an equal volume of sulfuric acid and shaken, the lard on separating has a brown-red tint.

Olive oil, with a few drops of sulfuric acid, gives a green color, while cottonseed, peanut and other vegetable oils, when thus treated with sulfuric and nitric acids, show brown to black coloration. The delicacy of the reaction may be increased by first dissolving the fat or oil in petroleum ether.

In the use of the coloration test with solvents a convenient method is to dissolve about one cubic centimeter of the fat in a test tube in petroleum ether, add one drop of strong sulfuric acid and shake.

In the case of lard, the color does not change or becomes yellow or red. Cottonseed oil, similarly treated, shows a brown or black color.⁶⁵

341. Coloration with Phosphomolybdic Acid.—Among the color tests, one which we have found of use is the coloration produced in certain oils, mostly of a vegetable origin, by phosphomolybdic acid.⁶⁶

The method of applying the test is extremely simple. A few cubic centimeters of the oil or melted fat are dissolved in an equal volume of chloroform, and a third volume of 10 per cent. phosphomolybdic acid added. The mouth of the test tube is closed with the thumb, and the whole is violently shaken. On being left in repose, the phosphomolybdic acid gathers at the top, and the coloration produced therein is easily observed. Cottonseed oil, peanut oil and cod liver oil give a beautiful green when

⁶⁵ Gantter, *Zeitschrift für analytische Chemie*, 1893, **32** : 303.

⁶⁶ Welmans, *Journal of the Society of Chemical Industry*, 1892, **11** : 548; 1894, **13** : 615.

treated in this way, which is turned to a blue on the addition of ammonia. Linseed oil gives a green color, but forms a kind of emulsion which obscures the color to some extent. The pure lards rendered in the laboratory give no coloration whatever to the reagent, but it retains its beautiful amber color in every case. Mixtures containing as little as 10 per cent. cottonseed oil and 90 per cent. lard, show a distinct greenish tint, while 20 per cent. cottonseed oil gives a distinct green. It is probable that different samples of cottonseed oil, refined to different degrees or in different ways, vary in their deportment with phosphomolybdic acid as they do with silver nitrate. In other words, there may be some samples of cottonseed oil which will not give the green color when treated as above, or so faintly as to have no diagnostic value in mixtures.

This reaction shows itself with nearly all vegetable oils but those which have been chemically treated either for the purpose of bleaching, or for the removal of the acidity, do not respond to the test at all, or else in a feeble manner, and that only after standing some time. Lard, goose fat, tallow, deer fat, butter fat, etc., show no change in color on being treated with this reagent, either with or without the addition of alkali. The presence of a small quantity of vegetable oil betrays itself by the appearance of the above mentioned coloration, the intensity of which forms an approximate measure of the amount of vegetable oil present in the sample. In experiments with suspected lards, which deviated in their iodine absorption numbers from those of genuine lard, the results were concordant, the color deepening as the iodine figure rose. The mineral fats (paraffin, vaselin) are without action on this reagent, and the only animal fat which reduces it is codliver oil.

In like manner samples of lard which have become rancid may be found which exhibit a deportment with this reagent similar to that shown with vegetable oils, and tallow and lard oil have been shown to give more distinct reactions than some of the vegetable oils.⁶⁷

⁶⁷ Lewkowitsch, *Chemical Technology and Analysis of Oils, Fats and Waxes*, 4th Edition, 1909, 1 : 401 ; 2 : 348.

Lewkowitsch, *Journal of the Society of Chemical Industry*, 1894, 13 : 619.

The phosphomolybdic acid may be prepared by precipitating a solution of ammonium molybdate with sodium phosphate and dissolving the washed precipitate in a warm solution of sodium carbonate. The solution is evaporated to dryness and the dry residue subjected to heat. If a blue coloration be produced it may be discharged by adding a little nitric acid and reheating. The residue is dissolved in water, acidified with nitric and made of such a strength as to contain about 10 per cent. of the substance.

342. Coloration with Picric Acid.—If to 10 cubic centimeters of oil a cold saturated solution of picric acid in ether be added and the latter be allowed to evaporate slowly, the acid remains dissolved in the oil, to which it communicates a brown color.

Pure lard, after the evaporation of the ether, appears of a citron-yellow color; if cottonseed oil be present, however, the mixture assumes a brown-red color.⁶⁸

343. Coloration with Silver Nitrate.—The use of silver nitrate in producing colorations in oils and fats has been proposed by Brullé.⁶⁹ The reagent employed consists of 25 parts of silver nitrate in 1,000 parts of alcohol of 95 per cent. strength. Twelve cubic centimeters of the oil to be examined and five of the reagent are placed in a test tube, held in a vessel containing boiling water, and the ebullition continued for about 20 minutes. At the end of this time an olive oil, even if it be an impure one, will show a beautiful green tint. With seed oils the results are quite different. Cotton oil submitted to this treatment becomes completely black. Peanut oil shows at first a brown-red coloration and finally a somewhat green tint, losing its transparency. Sesame oil is distinguished by a red-brown tint very pronounced and remaining red. Colza oil takes on a yellowish green coloration, becomes turbid and is easily distinguished in its reaction from olive oil. In mixtures of olive oil with the other oils, any notable proportion of the seed oils can be easily determined by the above reactions. Natural butter treated with this reagent retains its primitive color. That containing margarin becomes a brick-red

⁶⁸ Comptes Rendus, 112 : 105.

⁶⁹ Pharmaceutische Zeitung, 1891, 36 : 798 ; The Analyst, 1892, 17 : 59.

and as little as five per cent. of margarin in butter can be detected by this test. With 10 per cent. the tint is very pronounced.

344. Coloration with Stannic Bromid.—This reagent is prepared by adding dry bromin, drop by drop, to powdered or granulated tin held in a flask immersed in ice water, until a persistent red color indicates that the bromin is in excess. In the application of this reagent three or four drops of it are added successively to a little less than that quantity of the oil, the mixture well stirred and set aside for a few minutes. The unsaponifiable matters of castor oil give a green color when thus treated, sandal wood oil a blood-red color and cedar oil a purplish color.⁷⁰

345. Coloration with Auric Chlorid.—The use of auric chlorid for producing colorations in oils and fats was first proposed by Hirschsohn.⁷¹ One gram of auric chlorid is dissolved in 200 cubic centimeters of chloroform and about six drops of this reagent added to five cubic centimeters of the oil to be tested. In the case of cottonseed oil a beautiful red color is produced.

I have found that even pure lards give a trace of color sometimes with this reagent, and therefore the production of a slight red tint cannot in all cases be regarded as conclusive of the presence of cottonseed oil.⁷²

In general, it may be said that the color reactions with fats and oils have a certain qualitative and sorting value, and in any doubtful case they should not be omitted. Their value can only be established by comparison under identical conditions with a large number of fats and oils of known purity. The analyst must not depend too confidently on the data found in books, but must patiently work out these reactions for himself, remembering always that the colors are due to very small quantities of some substance peculiar to a particular oil, and that refining methods and natural changes due to aging may entirely remove these.

346. Cottonseed Oil, Bechi's Test.—Crude, fresh cottonseed oil, when not too highly colored, and generally the refined article,

⁷⁰ Pearmain and Moor, *The Analyst*, 1895, 20 : 174.

⁷¹ *Pharmaceutische Zeitschrift für Russland*, 1888, 27 : 723 ; *American Journal of Pharmacy*, 1889, 61 : 23.

⁷² *Division of Chemistry, Bulletin* 13, part 4, 1889 : 502.

may be distinguished from other oils by the property of reducing silver salts in certain conditions. The reaction was first noticed by Bechi and has been the subject of extensive discussions.⁷³

The process as proposed by Bechi has been modified in many ways but apparently without greatly improving it. It is conducted as follows:

(c) BECHI OR SILVER NITRATE TEST FOR COTTON-SEED OIL.—

(1) *Preparation of Reagent.*⁷⁴—Dissolve two grams of silver nitrate in 200 cubic centimeters of 95 per cent. alcohol and 40 cubic centimeters of ether, adding one drop of nitric acid.

(2) *Determination.*—Mix 10 cubic centimeters of oil or melted fat, 5 cubic centimeters of reagent, and 10 cubic centimeters of amyl alcohol in a test tube. The addition of amyl alcohol is not necessary, but is very convenient, as it dissolves the oils or fats, which then mix with the reagent much better. Divide, heat one-half in a boiling water bath for 10 minutes and compare with portion not heated. Any blackening due to reduced silver shows presence of cottonseed oil.

The heating is also accomplished in a small porcelain dish on which is often deposited a brilliant mirror of metallic silver. The white color of the porcelain also serves as a background for the observation of the coloration produced. In most instances a green color has been noticed after the reduction of the silver is practically complete. Unless cottonseed oil has been boiled or refined in some unusual way, the test, as applied above, is rarely negative. The reduction of the silver is doubtless due to some aldehydic principle, present in extremely minute quantities, and which may be removed by some methods of technical treatment. The silver nitrate test therefore is reliable when the reduction takes place, but the absence of a distinct reaction may not in all cases prove the absence of cottonseed oil.

⁷³ *Annali del Laboratorio Chimico*, 1891-92 : 197; *Division of Chemistry, Bulletin* 13, part 4, 1889 : 465; *Journal of Analytical Chemistry*, 1887, 1 : 449; 1888, 2 : 119, 275.

⁷⁴ *Pearmain and Moor, Allen's Commercial Organic Analysis*, 3d Edition, 2 (1) : 143; *Wesson, Journal American Chemical Society*, 1895, 17 : 724.

Other oils which have become rancid⁷⁵ and lards which have been steamed or heated at high temperature contain decomposition products which have a reducing action on silver nitrate. Some salad oils which contain no cottonseed oil, according to the Halphen test, give a brown coloration with the Bechi reagent and in some cases reduce silver. Such oils when purified give no reaction, and therefore the oils or fats should always be purified before testing. To effect this, heat from 20 to 30 grams of the sample on a water bath for a few minutes together with 25 cubic centimeters of 95 per cent. alcohol, shake thoroughly, decant as much of the alcohol as possible, wash with two per cent. nitric acid, and finally with water. Heating the oils or fats to 100° or simply washing with two per cent. nitric acid is not sufficient except in a few cases.

(e) BAUDOUIN TEST FOR SESAME OIL.—PROVISIONAL.—Dissolve 0.1 gram of finely powdered sugar in 10 cubic centimeters of hydrochloric acid (sp. gr. 1.20), add 20 cubic centimeters of the oil to be tested, shake thoroughly for a minute, and allow to stand. The aqueous solution separates almost at once. In the presence of even a very small admixture of sesame oil this is colored crimson. Some olive oils give a slight pink coloration with this reagent, but they are not hard to distinguish if comparative tests with sesame oil are made.

(f) VILLAVECCHIA⁷⁶ TEST FOR SESAME OIL.—PROVISIONAL.—Add two grams of furfural to 100 cubic centimeters of alcohol (95 per cent.) and mix thoroughly 0.1 cubic centimeter of this solution, 10 cubic centimeters of hydrochloric acid (sp. gr. 1.20), and 10 cubic centimeters of oil by shaking them together in a test tube. The same color is developed as when sugar is used, as in the Baudouin test. Villavecchia explained this reaction on the basis that furfural is formed by the action of levulose and hydrochloric acid and therefore substituted furfural for sucrose. As

⁷⁵ Wesson, *Journal American Chemical Society*, 1895, **17** : 724; Winton, *Connecticut Agricultural Experiment Station Report*, 1900, **2** : 143.

⁷⁶ Villavecchia and Fabris, *Journal Society Chemical Industry*, 1893, **12** : 67; 1894 **13** : 69. Benedikt and Lewkowitsch, *Oils, Fats, and Waxes*, p. 385.

furfural gives a violet tint with hydrochloric acid it is necessary to use the very dilute solution specified in the method.

347. Special Nitric Acid Test.—A special nitric acid test for cottonseed oil is made with nitric acid of exactly 1.375 specific gravity at 15°. This test is especially valuable in detecting cottonseed in olive oil. The operation is conveniently conducted by shaking together equal volumes of the oil and acid in a test tube until an intimate mixture or emulsion is secured. When any considerable quantity of cottonseed oil is present an immediate brown coloration is produced, from the intensity of which the relative proportion of cottonseed oil in the case of a mixture may be roughly approximated. When only a little cottonseed oil is present in the mixture, the test tube containing the reagents should be set aside for several hours before the final observation is made.

348. Milliau's Process.—Milliau has proposed the application of the silver salt directly to the free fat acids of the oil instead of to the oil itself.⁷⁷ About 15 cubic centimeters of the oil are saponified with alcoholic potash in the usual manner, 150 cubic centimeters of water added to the dish and the mixture boiled until the alcohol is evaporated. The fat acids are freed by the addition of decinormal sulfuric acid and as they rise to the surface in a pasty condition are removed with a spoon. The free acids are washed with distilled water. The water is drained off and the free acids dissolved in 15 cubic centimeters of 92 per cent. alcohol and two cubic centimeters of a three per cent. solution of silver nitrate. The test tube containing the mixture is well shaken and placed in a water-bath, out of contact with light, and left until about one-third of the alcohol is evaporated. Ten cubic centimeters of water are added, the heating continued for a few minutes and the color of the supernatant fat acids observed. The presence of cottonseed oil is revealed by the production of a lustrous precipitate which colors the fat acids black. In some cases the process of Milliau gives better results than the original

⁷⁷ Rapport présenté à l'Académie Sciences le 20 février, 1883; Milliau, *Analyse Chimique des Matières Grasses*, 1891: 17; Division of Chemistry, *Bulletin* 13, part 4, 1889: 466.

method of Bechi, but this is not always the case. It does away with the use of amyl alcohol and colza oil, but its manipulation is more difficult. In all doubtful cases the analyst should apply both methods.

The following modification of Williani method recommended by Torelli and Ruggeri⁷⁸ gives more delicate indications it is said than the original. Five grams of the liquid fatty acids are dissolved in 10 cubic centimeters of alcohol and one cubic centimeter of a five per cent. silver nitrate solution is added, and solution heated to 70°-80°. Cotton seed oil will reduce the silver immediately.

349. Halphen's Test.—Probably the best color reaction for cottonseed oil is Halphen's⁷⁹ test with a carbon bisulfid solution of sulfur in amyl alcohol. As used in common practice the details are as follows: Mix carbon bisulfid containing about one per cent. of sulfur in solution with an equal volume of amyl alcohol, then add one volume of the reagent to one of the oil under examination, and heat in a bath of boiling saturated brine for from one to two hours. In the presence of as little as one per cent. of cottonseed oil, a characteristic red or orange-red color is produced.

Lard and lard oil from animals fed on cottonseed meal will give a faint reaction; the fatty acids also give this reaction.

The depth of color is proportional, to a certain extent, to the amount of oil present, and by making comparative tests with cottonseed oil some idea as to the amount present can be obtained, but it must be remembered that different oils react with different intensities, and oils which have been heated from 200° to 210°, react with greatly diminished intensity. Heating 10 minutes at 250° renders cottonseed oil incapable of giving a reaction.⁸⁰

The nature of the chromogenetic substance to which this color is due has not been fully settled but is generally believed to be of an aldehydic nature. Dupont⁸¹ believed the darkening in the

⁷⁸ *Annali del Laborat. Centr. delle Gabelle*, 1900.

⁷⁹ *Zeitschrift für Untersuchung Nahrungs und Genussmittel*, 1898, 1: 81.

⁸⁰ Holde and Pelgry, *Journal Society Chemical Industry*, 1899, 18: 711

⁸¹ *Bulletin Society Chemistry*, 1895 [3] 696.

Becchi test due to the formation of silver sulfide as he found that by passing a current of steam through heated cottonseed oil a substance yielding sulfuric acid upon oxidation was obtained. Ficher and Peyau⁸² have confirmed the presence of this body. In any event oils previously heated to 250° C, or kept in the light for a long time fail to respond to either the Becchi or Halphen reactions, so that they are not absolutely reliable. It must also be remembered that lard and possibly other fats from animals fed on cottonseed meal⁸³ will give the Halphen reaction as also a few other oils as Kapok and Baobab.⁸⁴ For the detection of cottonseed oil in suspected samples of lard Tolman⁸⁵ recommends the separation of phytosterol when possible as the only safe method. He has shown that lards from cottonseed meal fed hogs give no characteristic crystals of phytosterol even when a Halphen test indicated as much as 15 per cent. of cottonseed oil, and on the other hand that as little as five per cent. of added oil could be detected even though it had been heated for half an hour at 260° and gave no longer a reaction with silver nitrate or Halphen's reagent.

350. Detection of Sesame Oil.—Milliau has pointed out a characteristic reaction of this oil which may be used with advantage in cases of doubtful identity.⁸⁶ The identification is based on the fact that the free acids of sesame oil, or some concomitant thereof, give a rose-red color like the oils themselves when brought in contact with a solution of sugar in hydrochloric acid.

The analytical process is conducted as follows: About 15 grams of the oil are saponified with alcoholic soda and when the reaction is complete treated with 200 cubic centimeters of hot water and boiled until the alcohol is removed. The fat acids are

⁸² *Zeitschrift für Untersuchung der Nahrungs und Genussmittel*, 1905, **9** : 81.

⁸³ *Zeitschrift für angewandte chemie*, 1901, **14** : 685; *Zeitschrift für offentl chemie*, 1901 : 140.

⁸⁴ *Journal American Chemical Society*, 1902, **24** : 1148; 1904, **26** : 837; 1906, 458.

Compt. rend., 1904, 807.

⁸⁵ *Journal of American Chemical Society*, 1905, **27** : 589.

⁸⁶ Milliau, *Analyse Chimique des Matières Grasses*, 1891 : 15.

set free with decinormal sulfuric acid and removed with a spoon as they rise to the surface in a pasty state, in which condition they are washed by shaking with water in a large test tube. When washed, the acids are placed in an oven at 105° until the greater part of the water is evaporated and the acids begin to become fluid. At this point they are treated with half their volume of hydrochloric acid saturated with finely ground sugar. On shaking the mixture, a rose color is developed which is characteristic of the sesame oil. Other oils give either no coloration or at most a yellow tint.

351. Thermal Reactions.—The measurement of the heat produced by mixing glycerids with reagents which decompose them or excite other speedy chemical reactions, gives valuable analytical data. These measurements may be made in any convenient form of calorimeter. The containing vessel for the reagents should be made of platinum or some other good conducting metal not affected by them.

The heat produced is measured in the usual way by the increment in temperature noted in the mass of water surrounding the containing vessel or the reaction mixture itself. The determination of the heat produced in chemical reactions is a tedious and delicate operation requiring special forms of apparatus for different substances, the time element in these operations is a matter of importance. Also it is necessary to work in rooms subject to slight changes of temperature and to leave the apparatus for some time at rest, in order to bring it and its contents to a uniform temperature. For these reasons the more elaborate methods of calorimetric examination are not well suited to ordinary analytical work, and the reader is referred to standard works on thermal chemistry for the details of such operations.⁸⁷ For our purpose here a description of a few simple thermal processes, easily and quickly conducted, will be sufficient, while a description of the method of determining the heat of combustion of foods will be given in another place.

⁸⁷ Muir, *Elements of Thermal Chemistry*, 1885 : 25 et. seq.

352. Heat of Sulfuric Saponification.—Maumené was the first to utilize the production of heat caused by mixing sulfuric acid with a fat as an analytical process.⁸⁸ In conducting the process a sulfuric acid of constant strength should be employed inasmuch as the rise of temperature produced by a strong acid is much greater than when a weaker acid is employed. The process is at best only comparative and it is evident that the total rise of temperature in any given case depends on the strength of the acid, the character and purity of the fat or oil, the nature of the apparatus and its degree of insulation, the method of mixing and the initial temperature. For this reason the data given by different analysts vary greatly.⁸⁹ For some of the methods of conducting the operation the reader may consult the work of Allen, cited above, or other authorities.⁹⁰

The process is conducted as follows:⁹¹ The initial temperature of the reagents should be a constant one. For oils 20° is a convenient starting point and for fats about 35°, at which temperature most of them are soft enough to be easily mixed with the reagent. The acid employed should be the pure monohydrated form, specific gravity at 20°, 1.845.

The apparatus used is shown in Fig. 91.

The test-tube which holds the reagents is 24 centimeters in length and five in diameter. It is provided with a stopper having three perforations, one for a delicate thermometer, one for a bulb funnel for delivering the sulfuric acid, and one to guide a stirring rod bent into a spiral as shown. The thermometer is read with a magnifying glass. Fifty cubic centimeters of the fat are placed in the test-tube and 10 of sulfuric acid in the funnel and the apparatus is exposed at the temperature desired until all parts of it, together with the reagents, have reached the same degree. The test-tube holding the oil should be placed in a vacuum-jacket

⁸⁸ *Comptes rendus*, 1852, **35** : 572.

⁸⁹ Allen, *Commercial Organic Analysis*, 3rd Edition, 1899, **2** : 76.

⁹⁰ Lewkowitsch, *Chemical Technology and Analysis of Oils, Fats, Waxes*, 4th Edition, 1909 : Vol. I, 385.

Jean, *Chimie Analytique des Matières Grasses*, 1892 : 217.

⁹¹ Bureau of Chemistry, *Bulletin* 107 (revised), 1910 : 143.

tube, such as will be described on the next page. The oil is allowed to run in as rapidly as possible from the funnel and the stirring rod is moved up and down two or three times until the oil and acid are well mixed. Care must be exercised to stir no more than is necessary for good mixing. The mercury is

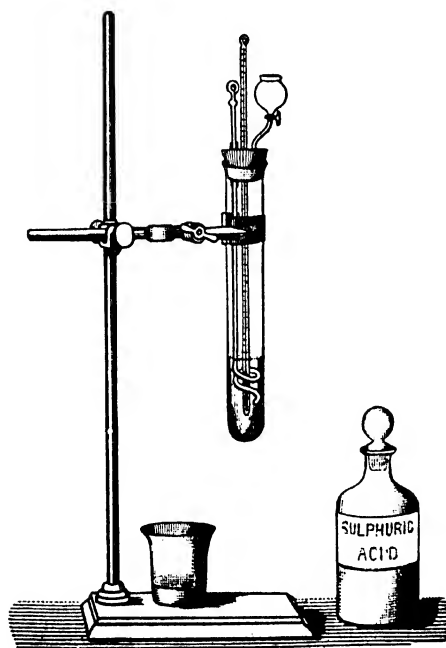


Fig. 91.—Apparatus for Determining the Rise of Temperature with Sulfuric Acid.

observed as it ascends in the tube of the thermometer and its maximum height is noted. With the glass it is easy to read to tenths, when the thermometer is graduated to fifths of a degree. When oils are tested which produce a rise of temperature approaching 100° , in the above circumstances (cottonseed, linseed and some others), either small quantities should be used or the

oil diluted with some inert substance or dissolved in some inert solvent of high boiling point.

Provisional Method of the Association of Official Agricultural Chemists.—The official chemists have modified the above method by using jacketed beakers which prevent undue radiation of heat.⁹²

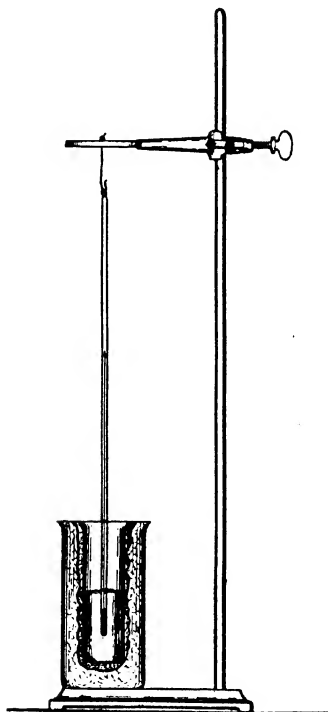


Fig. 91a.—Apparatus for Determining Rise of Temperature.

Place a beaker, 12.7 by 3.8 cubic centimeters inside of another 15.2 by 7.6 cubic centimeters and pack a wet mixture of asbestos and plaster of Paris tightly around the inner beaker. This, when dried, makes a hard, solid packing which radiates heat very slowly. The apparatus is illustrated in Fig. 91a.

⁹² Bureau of Chemistry, Bulletin 107 (revised), 1912 : 143.

Remove the inner beaker, place in it 50 grams of fat, and note the temperature carefully. Then from a pipette which will deliver it in approximately one minute add 10 cubic centimeters of the strongest sulfuric acid which is at the same temperature as the oil. Stir the oil and acid with an accurate thermometer while the acid is being introduced, then hold the thermometer bulb carefully in the center of the mixture, and when the mercury reaches the highest point note the reading. It is easy to determine this point, as the column of mercury remains stationary for some time. Do not read the temperature too soon, as some oils take considerable time to reach their maximum point.

The difference between the initial and the final reading expressed in degrees centigrade is the Maumené number.

Great care must be taken to use acid of maximum strength. With the semi-drying oils, such as cottonseed, the use of this strength of acid will cause foaming and make it almost impossible to obtain the correct rise of temperature. With such oils either a weaker acid must be used and the results compared with the rise of temperature with water or a dilution with paraffin oil is made. It is always best to test the apparatus and acid by use of water and oils of known purity. In reporting results, the rise of temperature with water should be stated, otherwise they possess no comparative value.

For a study of the variations produced in the rise of temperature when varying proportions of oil and acid are used, the work of Munroe may be consulted.⁹³

The thermoleometer described by Jean is a somewhat complicated piece of apparatus and does not possess any advantage over the simple form described above.⁹⁴

Instead of expressing the data obtained in thermal degrees showing the rise of temperature, Thompson and Ballantyne refer them to the heat produced in mixing sulfuric acid and water.⁹⁵

The observed thermal degree of the oil and acid divided by

⁹³ Division of Chemistry, Bulletin 13, part 4, 1889 : 445 ; Proceedings American Public Health Association, 1884, 10 : 236.

⁹⁴ Jean, *Chimie Analytique des Matières Grasses*, 1892 : 61.

⁹⁵ Journal of the Society of Chemical Industry, 1891, 10 : 234.

that of the water and acid is termed the specific temperature reaction. For convenience in writing, this quotient is multiplied by 100. The respective quantities of acid and water are 10 and 50 cubic centimeters. This method of calculation has the advantage of eliminating to a certain degree the variations which arise in the use of sulfuric acid of differing specific gravities. In the following table are given the comparative data obtained for some common oils.⁹⁶

Kind of oil	Acid of 95.4 per cent.		Acid of 96.8 per cent.		Acid of 99 per cent.	
	Rise of temperature with the oil °°	Specific temperature reaction °°	Rise of temperature with the oil °°	Specific temperature reaction °°	Rise of temperature with the oil °°	Specific temperature reaction °°
Olive oil	36.5	95	39.4	85	44.8	96
Rapeseed oil	49.0	127	37.0	89	58.0	124
Castor oil	34.0	88
Linseed oil	104.5	270	125.2	269

353. Method of Richmond.—The rise of temperature produced by mixing an oil and sulfuric acid is determined by Richmond in a simple calorimeter, which is constructed by fitting a small deep beaker inside a larger one with a packing of cotton. The heat capacity of the system is determined by adding to 10 grams of water, in the inner beaker, at room temperature, 25 grams of water of a noted higher temperature and observing the temperature of the mixture. The cooling of the system, during the time required for one determination of heat of sulfuric saponification, does not exceed one per cent. of the whole number of calories produced.⁹⁷ Between the limits of 92 per cent. and 100 per cent. the rise of temperature observed is directly proportional to the strength of the acid.

Relative Maumené Figure.—The total heat evolved per mean molecule is called by Richmond the relative maumené figure. It is calculated as follows:

⁹⁶ Lewkowitsch, *Chemical Technology and Analysis of Oils, Fats, Waxes*, 4th Edition, 1909 : Vol. I, 391.

See also Jenkins *Journal of the Society of Chemical Industry*, 1897, 194.

⁹⁷ *The Analyst*, 1895, 20 : 58.

Let x = percentage of sulfuric acid in the acid employed;
 h = heat capacity of calorimeter in grams of water;
 R = observed rise of temperature (25 grams of oil, five cubic centimeters sulfuric acid);
 K = potash absorbed for saponification (19.5 per cent. of potassium hydroxid, standard of comparison);
 M = relative Maumené figure:

$$\text{Then } M = R \times \frac{21.5}{x-78.5} \times \frac{20+h}{20} \times \frac{19.5}{K}$$

Recently Tortelli⁹⁸ has published the results of an exhaustive investigation on the thermal indices of various fats, giving the figure which he obtained, using his thermoleometer. This is a vacuum jacketed beaker and thermometer equipped with wings to assist in stirring the reaction mixture. Twenty cubic centimeters of the oil to be tested are stirred in the beaker with the thermometer and the temperature noted, then five cubic centimeters of H_2SO_4 (sp. gr. 1.8413 at 15°) gradually added and the final reading made when the temperature has remained constant for two minutes. The rise is designated the *thermal number*. By this method very definite values are found for each oil, olive giving 44° , a figure much lower than that for cottonseed and other common adulterants. In the determination of the thermal number for oils high in unsaturated fatty acids, *i. e.*, linseed, and train-oil, that give a value exceeding 90° , the sample is diluted with a definite volume of olive rather than paraffin or other mineral oils as these give erroneous results. The thermoleometer may be used for determining the thermal value of solid fats by taking into account the specific heat of the fat and acid and using the formula derived as follows:

Q , = amount of H_2SO_4 ,
 C , = specific heat of the acid,
 t , = the original temperature,
 Q_o = grams of fat,
 C_o = specific heat of fat,
 t_o = temperature at moment of adding acid,
 t = thermal number.

⁹⁸ *Chemiker Zeitung*, 33 : 125, 134, 171, 184, 389.
Annales des falsifications, 2 : 176, 280.

$$\text{Then } t = \frac{(C_s t_s + C_o t_o)}{(C_s + C_o)}.$$

Tortelli maintains that the determination of the thermal number may be substituted for the iodine determination and the iodine value calculated by factors which he has derived experimentally, but as these vary from 1.84 for non-drying oils such as olive oil, 1.60 for the semi-drying to 1.48 for the drying oils, this statement should be taken with considerable caution.

354. Heat of Bromination.—The rise of temperature caused by mixing fats with sulfuric acid has long been used to discriminate between different fats and oils. Lehner and Mitchell propose a similar reaction based upon the rise of temperature produced by mixing bromine with the sample.⁹⁹ The action of bromine on unsaturated fatty bodies is instantaneous and is attended with a considerable evolution of heat. Since the action of bromine on many of the oils is very violent it is necessary to dilute the reagent with chloroform or glacial acetic acid. Owing to its high boiling point the acetic acid has some advantage over chloroform for this purpose. The tests are conveniently made in a vacuum-jacket tube. In such a tube there is no loss of heat by radiation. The bromine is measured in a pipette having at its upper end a tube filled with caustic lime held between plugs of asbestos. The bromine sample to be tested and the diluent employed are kept at the same temperature before beginning the trial. They are quickly mixed and the rise of temperature noted. The oil is first dissolved in the chloroform and the bromine then added.

A somewhat constant relation is noticed between the rise of temperature and the iodine number when one gram of oil, 10 cubic centimeters of chloroform and one cubic centimeter of bromine are used.

If the rise in temperature in degrees be multiplied by 5.5 the product is approximately the iodine number of the sample. Thus a sample of lard gave a rise in temperature of 10°.6 and an iodine number of 57.15. The number obtained by multiplying 10.6 by 5.5 is 58.3.

⁹⁹ The Analyst, 1895, 20 : 146.

In like manner the numbers obtained for some common oils are as follows:

Material	Rise of temperature with bromin	Iodin No	Calculated iodin No
Butter fat.....	6.6	37.1	36.3
Olive oil	15.0	80.8	82.5
Maize "	21.5	122.0	118.2
Cotton "	19.4	107.1	106.7
Castor "	15.0	83.8	82.5
Linseed oil	30.4	160.7	167.2
Codliver "	28.0	144.0	140.0

355. Modification of the Heat of Bromination Method.—The method described above by Helmer and Mitchell presents many grave difficulties in manipulation, on account of the inconvenience of handling liquid bromin. The process is made practicable by dissolving both the oil or fat and the bromin in chloroform, or better in carbon tetrachlorid, in which condition the bromin solution is easily handled by means of a special pipette.¹

In order to make a number of analyses of the same sample 10 grams of the fat may be dissolved in chloroform or carbon tetrachlorid and the volume completed with the same solvent to 50 cubic centimeters. In like manner 20 cubic centimeters of the bromin are dissolved in one of the solvents named and the volume completed to 100 cubic centimeters therewith.

For convenience of manipulation the solutions are thus made of such a strength that five cubic centimeters of each represent one gram of the fat and one cubic centimeter of the liquid bromin respectively.

The apparatus used for the work is shown in the accompanying figure. The pipette for handling the bromin solution is so arranged as to be filled by the pressure of a rubber bulb, thus avoiding the danger of sucking the bromin vapor into the mouth. The filling is secured by keeping the bromin solution in a heavy Erlenmeyer with a side tubulure such as is used for filtering under pressure. The solutions are mixed in a long tube, held in a larger vessel, from which the air is exhausted to secure a minimum radiation of heat. A delicate thermometer graduated

¹ Journal of the American Chemical Society, 1896, 18 : 378.

in tenths serves to register the rise of temperature. The fat solution is first placed in the test tube, with care not to pour it down the sides of the tube but to add it by means of a pipette reaching nearly to the bottom. The whole apparatus having been allowed to come to a standard temperature the bromin solution is allowed

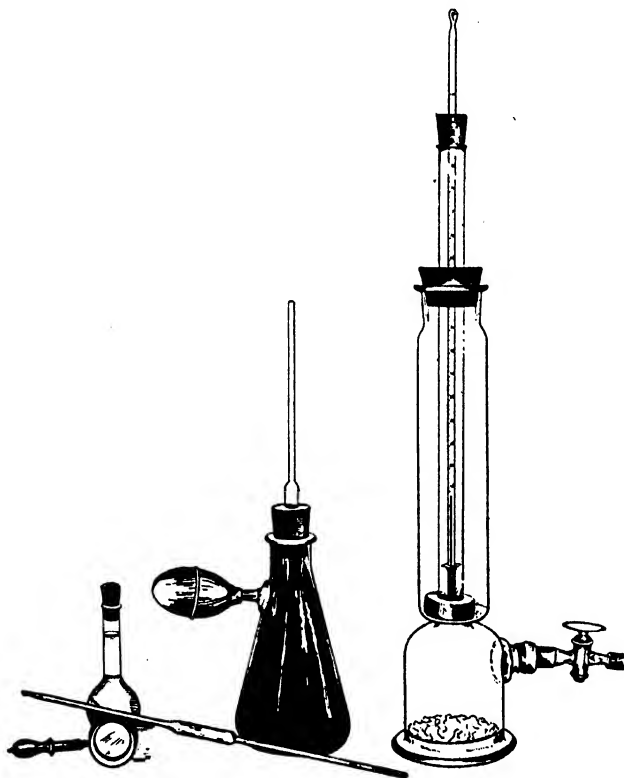


Fig. 92.—Apparatus for Determining Heat of Bromination.

to run in quickly from the pipette. No stirring is required as the liquids are sufficiently mixed by the addition of the bromin solution. The mercury in the thermometer rapidly rises and is read at its maximum point by means of a magnifying glass. With a

thermometer graduated in tenths, it is easy to read to twentieths of a degree.

It is evident that the rise of temperature obtained depends on similar conditions to those mentioned in connection with sulfuric saponification. Each system of apparatus must be carefully calibrated under standard conditions and when this is done the comparative rise of temperature obtained with various oils and fats will prove of great analytical use. It is evident that the ratio of this rise of temperature to the iodine number must be determined for every system of apparatus and for every method of manipulation employed, and no fixed factor can be given that will apply in every case.

With the apparatus above described and with the method of manipulation given the following data were obtained for the oils mentioned:

	Rise of temperature
Olive oil	20°.5
Refined cottonseed oil	25°.7
Sunflowerseed oil	28°.4
Calycanthusseed oil	29°.0

Bromine and chloroform, when mixed together, give off heat, due to the chemical reaction resulting from the substitution of bromine for hydrogen in the chloroform molecule and the formation of hydrobromic acid. For this reason the data obtained, when chloroform is used as a solvent, are slightly higher than with carbon tetrachloride. The use of the latter reagent is therefore to be preferred.

356. Consistence.—It has already been said that oils are mostly of vegetable origin and the solid fats of animal derivation. In the animal economy it would be a source of disturbance to have in the tissues a large body of fat which would remain in a liquid state at the normal temperature of the body. Nearly all the animal fats are found to have a higher melting point than the body containing them. An exception is found in the case of

butter fat, but it should be remembered that this fat is an excretion and not intended for tissue building until it has undergone subsequent digestion. Fish oils are another notable exception to the rule, but in this case these oils can hardly be regarded as true glycerids in the ordinary sense of that term.

In general, it may be said that a sample of a glycerid, which in its natural state remains liquid at usual room temperatures, is probably an oil of vegetable origin. Fish oils have an odor and taste which prevent them from being confounded with vegetable oils. In oils which are manufactured from animal glycerids such as lard oil, the discrimination is more difficult but peculiarities of taste and color are often perceptible.

357. Nature of the Fat Acid.—When it is not possible to discriminate between samples by the sensible physical properties just described, much light can be thrown on their origin by the determination of their other physical properties, such as specific gravity, refractive index, melting point, etc., in the manner already fully described. Further light may be furnished by saponification and separation of the fat acids. The relative quantities of oleic, stearic, palmitic, and other acids will help to a correct judgment in respect of the nature of the sample. The vegetable oils and lard oils, for instance, consist chiefly of olein; lard and tallow contain a large proportion of stearin; palm oil and butter fat contain considerable portions of palmitin, and the latter is distinguished moreover by the presence of soluble and volatile acids combined as butyrin and its associated glycerids.

Oleic acid can be rather readily separated from stearic and palmitic by reason of the solubility of its lead salts in ether. One method of accomplishing this separation has already been described (paragraph 339).

358. Separation with Lime.—A quicker, though perhaps not as accurate a separation of the oleic from the palmitic and stearic acids, is accomplished by means of lime according to the method developed by Bondzyuski and Ruff.² This process is used chiefly, however, to separate the free fat acids (palmitic, stearic)

² *Zeitschrift für analytische Chemie*, 1890, 29 : 4.

from the neutral fat and the free oleic acid. It probably has no point of superiority over the lead process.

359. Separation of the Glycerids.—The fact that olein is liquid at temperatures allowing palmitin and stearin to remain solid, permits of a rough separation of these two classes of bodies by mechanical means. The mixed fats are first melted and allowed to cool very slowly. In these conditions the stearin and palmitin separate from the olein in a crystalline form and the olein is removed by pressure through bags. In this way lard is separated into lard oil, consisting chiefly of olein, and lard stearin, consisting largely of stearin. Beef (caul) fat is in a similar manner separated into a liquid (oleo-oil) and a solid (oleo-stearin) portion. It is evident that these separations are only approximate, but by repeated fractionations a moderately pure olein or stearin may be obtained.

360. Separation as Lead Salts.—Muter's process, with a special piece of apparatus, has already been described (378). For general analytical work the special tube may be omitted. In a mixture of insoluble free fat acids all are precipitated by lead acetate, and the resulting soap may be extracted with ether, either with successive shaking or in a continuous extraction apparatus. In this latter case a little of the lead stearate or palmitate may pass into solution in the hot ether and afterwards separate on cooling. When the operation is conducted on from two to three grams of the dry mixed acids, the percentage proportions of the soluble and insoluble acids (in ether) can be determined. The lead salt which passes into solution can be decomposed and the oleic acid removed, dried and weighed. Dilute hydrochloric acid is a suitable reagent for decomposing the lead soap. The difference between the weight of the oleic acid and that of the mixed acids before conversion into lead soap furnishes the basis for the calculation. For further details in respect of the fat acids the reader may consult analytical work.³

361. Separation of Arachidic Acid.—Peanut oil is easily distinguished from other vegetable glycerids by the presence of arachidic acid, which is separated as follows:—

³ Allen, *Commercial Organic Analysis*, 3rd Edition, 1899, 2 : .

The method used by many chemists for separating arachidic acid is a modification of the usual methods based on the process as carried out by Milliau.⁴ About 20 grams of the oil are saponified with alcoholic soda, using 20 cubic centimeters of 36° baumé soda solution diluted with 100 cubic centimeters of 90 per cent. alcohol. When the saponification is complete, the soda is converted into the lead soap by treatment with a slight excess of a saturated alcoholic solution of lead acetate. Good results are also obtained by using dilute alcohol, viz., 50 per cent., instead of 90 per cent., in preparing the lead acetate solution.

While still warm the supernatant liquid is decanted, the precipitate washed by decantation with warm 90 per cent. alcohol and triturated with ether in a mortar four times, decanting the ethereal solution in each instance. By this treatment all of the lead oleate and hypogaeate are removed and are found in the ethereal solution, from which they can be recovered and the acids set free by hydrochloric acid and determined in the usual way.

The residue is transferred to a large dish containing two or three liters of pure water and decomposed by the addition of about 50 cubic centimeters of strong hydrochloric acid. The lead chlorid formed is soluble in the large quantity of water present, which should be warm enough to keep the free acids in a liquid state in which form they float as a clear oily liquid on the surface. The free acids are decanted and washed with warm water to remove the last traces of lead chlorid and hydrochloric acid. The last traces of water are removed by drying in a thin layer in vacuo. Practically all of the acids, originally present in the sample except oleic and hypogaeic, are thus obtained in a free state and their weight is determined.

The arachidic acid may be separated almost quantitatively by dissolving the mixed acids in 40 cubic centimeters of 90 per cent. alcohol, adding a drop of hydrochloric acid, cooling to 16° and allowing to stand until the arachidic acid has crystallized. The crystals are purified by washing twice with 20 cubic centimeters of 90 per cent. and three times with the same quantity of 70 per

⁴ Milliau, *Analyse Chimique des Matières Grasses*, 1891 : 13.

cent. alcohol. The residual impure arachidic acid is dissolved in boiling absolute alcohol, poured through a filter and washed with pure hot alcohol. The filtrate is evaporated to dryness and heated to 100° until a constant weight is obtained. From the above data, the percentages of oleic, hypogaeic, arachidic and other acids in the sample examined are calculated.

In the above process, owing to the pasty state of the lead soaps, the trituration in a mortar with ether is found troublesome. The extraction of the lead oleate and hypogaeate is facilitated by throwing the pasty ethereal mass on a filter and washing it thoroughly with successive portions of about 50 cubic centimeters of ether. By this variation, it was found by Krug under my direction, that less ether was required and a more complete removal of the lead oleate effected. The solution of the lead oleate is completed by about half a dozen washings with ether as above described. The extraction may also be secured by placing the lead soaps in a large extracting apparatus and proceeding as directed in paragraph 40. The residue is washed from the filter paper into a large porcelain dish and decomposed as already described with hydrochloric acid. After the separation is complete, the mixture is cooled until the acids are solid. The solid acids are then transferred to a smaller dish, freed of water and dissolved in ether. The ethereal solution is washed with water to remove any traces of lead salt or of hydrochloric acid. After the removal of the ether, the arachidic acid is separated as has already been described.

362. The Sulfur Chlorid Reaction.—Some vegetable oils, when treated with sulfur chlorid, give a hard product similar to elaidin, while lard does not. This reaction is therefore helpful in discriminating between some vegetable and animal glycerids. The process which is described by Warren has been used with some satisfaction.⁵

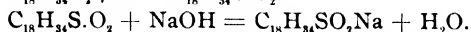
Five grams of the oil or fat are placed in a tared porcelain dish and treated with two cubic centimeters of carbon bisulfid and the

⁵Chemical News, 1888:113; Division of Chemistry. Bulletin 13, part 1, 1889:468.

same quantity of sulfur chlorid. The dish is placed on a steam-bath and its contents stirred until the reaction is well under way. The heating is continued until all volatile products are evaporated, the hard mass being well rubbed up to facilitate the escape of imprisoned vapors. The powdered or pasty mass is transferred to a filter and washed with carbon bisulfid to remove all unaltered oil. The washing with carbon bisulfid is hastened by pressure and about 200 cubic centimeters of the solvent should be used. After drying, the weight of insoluble matter is obtained and deducted from the total weight of the sample used.

The color and tenacity of the hard, insoluble portion are characteristic. The quantitative part of the operation appears to have but little value, but applied qualitatively as outlined above it produces hard, leathery masses with cotton, olive and peanut oils, and but little change in lard and beef fats. When thus applied, the process is conducted as described above but without making the weighings. In this instance it is as easy of application as the process of Bechi and is deserving of greater attention than has been given it by analysts.

In the combination which takes place between the sulfur and the fat it is probable that only addition products are formed, since the quantity of alkali required for saponification is not diminished by previously treating the fat with sulfur chlorid.⁶ The reactions which take place are probably well represented by the following equations, in which oleic acid is treated with sulfur chlorid:



363. Detection of Cholesterin and Phytosterin in Glycerids.—

Cholesterin is often found in animal glycerids and a corresponding body, phytosterin, is sometimes found in oils of a vegetable origin.⁷ When one of these two bodies is present it may be useful in distinguishing between animal and vegetable glycerids.

⁶ Zeitschrift für angewandte chemie, 1895, : 535.

⁷ Justus Leibig's Annalen des chemie, , 192 : 178; Zeitschrift für analytische chemie, 1887, 26 : 572; Division of Chemistry, Bulletin 13, part 4, 1889 : 514.

They are detected as follows: Fifty grams of the glycerids in each case are saponified with alcoholic alkali, preferably potash, in order to have a soft soap. After saponification is complete, the alcohol is evaporated and the residual soap dissolved in two liters of water. The mixture is shaken with ether and the ethereal solution evaporated to a small bulk. The residue, which may contain a small quantity of unsaponified fat is again treated with alcoholic potash and subjected a second time to the action of ether, as indicated above, with the addition of a few drops of water and of alcohol if the emulsion separate slowly. The ethereal extract finally secured is allowed to evaporate slowly and the cholesterin (phytosterin) is obtained in a crystalline form. The melting point of the cholesterin crystals is 146° and that of the phytosterin 132° .

Cholesterin crystallizes in thin rhombic tables while phytosterin separates in stellar aggregates or in bundles of long needles.

When dissolved in chloroform the two products show different color reactions with sulfuric acid, cholesterin giving a cherry and phytosterin a blue-red tint. In a mixture of animal and vegetable glycerids the two products are obtained together and the melting point of the mixture may afford some idea of the relative quantities of each present. It is evident, however, that no reliable judgment can be formed from these data of the relative proportions of the two kinds of glycerids in the original sample.

364. Cholesterin and Paraffin in Ether Extracts.—In ethereal extracts of some bodies, especially of flowers of the *crysanthemum*, paraffin is found combined with cholesterin. The two bodies may be separated as follows:*

The ether extract is treated with aqueous then with alcoholic potash several times; the residue soluble in ether is a solid body melting at from 70° to 100° .

If the ethereal solution be cooled in a mixture of snow and salt, a crystalline deposit is formed. This substance, purified by repeated precipitations, is obtained colorless in fine crystalline scales melting at 64° . It is very soluble in ether, benzene

* *Journal de Pharmacie et de chimie*, 1889, : 447.

and chloroform almost insoluble in cold alcohol, and somewhat soluble in hot.

Its percentage composition is :

	Per cent.
Carbon	85.00
Hydrogen	14.95

It is therefore a paraffin.

The ethereal solution, freed by the above process from paraffin, leaves on evaporation a crystalline mass which is cholesterin, retaining still a small quantity of fat matters. In treating the crystals with alcoholic potash these fat bodies are saponified and the residue is taken up with ether. The cholesterin is obtained in fine needles melting at from 170° to 176°. It presents all the reactions of cholesterin, especially the characteristic reaction with chloroform and sulfuric acid.

365. Absorption of Oxygen.—Among oils a distinction is made between those which oxidize readily and those which are of a more stable composition. Linseed oil, for instance, in presence of certain metallic oxides, absorbs oxygen readily and is a type of the drying oils, while olive oil represents the opposite type.

The method of determining the quantity of oxygen absorbed is due to Livache and is carried out as follows :⁹

Precipitated metallic lead (by zinc) is mixed in a flat dish, with the oil to be tested, in the proportions of one gram of lead to three-quarters of a gram of oil, and exposed to the air and light of the workroom. The dish is weighed from time to time until there is no longer any increase in weight.

Instead of lead, finely divided copper has been used by Krug under my direction, but the percentage of absorption of oxygen is not so high with copper as with lead. Krug found the quantities of oxygen absorbed, after nine days, by the samples treated with copper and lead respectively to be the following:

	Copper, per cent oxygen ab- sorbed	Lead, per cent oxygen absorbed
Olive oil	1.69	2.03
Cottonseed oil.....	4.25	5.30
Peanut oil.....	2.74	3.87
Linseed oil.....	5.55	7.32

⁹ Journal Society of Chemical Industry, 1886 : 494. Comptes rendus, 1886 : 12 : 1167.

Livache found that linseed oil absorbed about twice as much oxygen as indicated by the data just given.

366. Elaidin Reactions.—In discriminating between oils and fats having a preponderance of olein and others with a smaller proportion of that glycerid, the conversion of the olein into its isomer elaidin is of diagnostic value. The following will be found a convenient method of applying this test.¹⁰

About 10 cubic centimeters of the oil are placed in a test tube together with half that quantity of nitric acid and one gram of mercury. The mixture is shaken until the mercury dissolves when the mass is allowed to remain at rest for 20 minutes. At the end of this time it is again shaken and placed aside. In from one to three hours the reaction is complete. Olive, peanut and lard oils give very hard elaidins. The depth to which a plunger of given weight and dimensions sinks into an elaidin mixture at a given temperature, has been used as a measure of the percentage of olein contained in the sample of oil, but it is evident that such a determination is only roughly approximate. Copper may be used instead of mercury for the generation of the oxids of nitrogen, but it is not so effective. The vapors of nitric oxids may also be conducted directly into the oil from a convenient generator. The reaction may also be accomplished by shaking the oil with nitric acid and adding, a drop at a time, a solution of potassium nitrite.

366a. Renard's Test* for Peanut Oil as Modified by Tolman.—Weigh 20 grams of oil into an Erlenmeyer flask. Saponify with alcoholic potash, neutralize exactly with dilute acetic acid, using phenolphthalein as indicator, and wash into a 500 cubic centimeters flask containing a boiling mixture of 100 cubic centimeters of water and 120 cubic centimeters of a 20 per cent. lead acetate solution. •Boil for a minute and then cool the precipitated soap by immersing the flask in water, occasionally giving it a whirling motion to cause the soap to stick to the sides of the flask. After the flask has cooled, the water and excess of lead

¹⁰ Division of Chemistry, Bulletin 13, part 4, 1889 : 515.

* Comptes rendus, 1871, 73 : 1330; Lewkowitsche, Oils, Fats, and Waxes, 4th Edition, 2 : 252.

- can be poured off and the soap washed with cold water and with 90 per cent. (by volume) alcohol. Add 200 cubic centimeters of ether, cork, and allow to stand for some time until the soap is disintegrated; heat on the water bath, using a reflux condenser, and boil for about five minutes. In the oils most of the soap will be dissolved, while in lards, which contain much stearin, part will be left undissolved. Cool the ether solution of soap to from 15° to 17° and let stand until all the insoluble soaps have crystallized out (about twelve hours).

Filter and thoroughly wash the precipitate with ether. Save the filtrate for the determination of the iodine number of the liquid fatty acids by the Muter method. Wash the soap on the filter back into the flask by means of a stream of hot water acidified with hydrochloric acid. Add an excess of dilute hydrochloric acid, partially fill the flask with hot water, and heat until fatty acids form a clear oily layer. Fill the flask with hot water, allow the fatty acids to harden and separate from the precipitated lead chlorid, wash, drain, repeat washing with hot water, and dissolve the fatty acids in 100 cubic centimeters of boiling 90 per cent. by volume alcohol. Cool to 15° , shaking thoroughly to aid crystallization.

From five to 10 per cent. of peanut oil can be detected by this method, as it effects a complete separation of the soluble acids from the insoluble, which interfere with the crystallization of the arachidic acid. Filter, wash the precipitate twice with 10 cubic centimeters of 90 per cent. by volume alcohol, and then with alcohol 70 per cent. by volume. Dissolve the precipitate with boiling absolute alcohol, evaporate to dryness in a weighed dish, dry, and weigh. Add to this weight 0.0025 gram for each 10 cubic centimeters of 90 per cent. alcohol used in the crystallization and washing if done at 15° C.; if done at 20° add 0.0045 gram for each 10 cubic centimeters. The melting point of arachidic acid thus obtained is between 71° and 72° . Twenty times the weight of arachidic acid will give the approximate amount of peanut oil present. No examination for adulterants in olive oil is complete without making the test for peanut oil.

Arachidic acid crystals have a characteristic structure and can be detected by the microscope.

The melting point of pure arachidic acid varies from 73° to 75° .

366b. Identification of Oils and Fats.—Properly, the methods of identifying and isolating the different oils and fats should be looked for in works on food adulteration. There are, however, many characteristics of these glycerids which can be advantageously discussed in a work of this kind. Many cases arise in which the analyst is called upon to determine the nature of a fat and discover whether it be admixed with other glycerids. It is important often to know in a given case whether an oil be of animal or vegetable origin. Many of the methods of analysis already described are found useful in such discriminations. For instance, a large amount of soluble or volatile acids in the sample under examination, would indicate the presence of a fat derived from milk while the form of the crystals in a solid fat would give a clue to whether it were the product of the ox or the swine. In the succeeding paragraphs will be briefly outlined some of the more important additional methods of determining the nature and origin of fats and oils of which the history is unknown.

The data obtained by means of the methods which have been described, both physical and chemical, are all useful in judging the character and nature of a glycerid of unknown origin. The colorations produced by oxidizing agents, in the manner already set forth will be found useful, especially when joined to those obtained with cottonseed and sesame oils yet to be described. For instance, the red coloration produced by nitric acid of 1.37 specific gravity is regarded by some authorities as characteristic of cottonseed oil as well as the similar coloration with Halphen's reagent. The coloration tests with silver nitrate (paragraph 343) and with phosphomolybdic acid (paragraph 341) are also helpful in classifying oils in respect of their animal or vegetable origin. The careful consideration of these tests, together with a study of the numbers obtained by treating the samples with iodine, with alcoholic KOH, and the heat of bromination and sulfuric saponification, is commended to all who are interested in classify-

ing oils. In addition to these reactions a few specific tests are added for more detailed work.

Chemical Constants.—Inasmuch as the so-called chemical constants are most valuable in separating the oils into broad groups and thus narrowing down the scope of the special tests which it is necessary to apply to a sample, they will be taken up first and treated in the order of their apparent importance.

367. Haloid Addition Numbers.—Many of the glycerids possess the property of combining directly with the haloids and forming thereby compounds in which the haloid, by simple addition, has become a part of the molecule. Olein is a type of this class of unsaturated glycerids. The process may take place promptly as in the case of bromin or move slowly as with iodin. The quantity of the haloid absorbed is best determined in the residual matter and not by an examination of the fat compound. By reason of the ease with which the amount of free iodine in solution can be determined, this substance is the one which is commonly employed in practical work.

In general, the principle of the operation depends on bringing the fat and haloid together in a proper solution and allowing the addition to take place by simple contact. The quantity of the haloid in the original solution being known, the amount which remains in solution after the absorption is complete, deducted from that originally present, will give the quantity which has entered into combination with the glycerid.

Inasmuch as the determination of the iodine value of a sample gives more information than any other single test, concerning the general class to which the oil belongs, *i. e.*, drying, semi-drying or non-drying, and is readily made, it may be considered the most important. The age of the product does not materially affect this factor unless some oxidation has occurred, and especially in the case of drying oils, it is a most useful determination as showing the degree of drying that has already or may be expected to take place with the sample in question.

368. Hübl's Process.—In determining the quantity of iodine which will combine with a fat, the method first proposed by

Hübl, or some modification thereof, is universally employed.¹¹ In the determination of the iodine number of a glycerid it is important that it be accomplished under set conditions and that iodine be always present in large excess. It is only when data are obtained in the way noted that they can be regarded as useful for comparison and determination. Many modifications of Hübl's process have been proposed, but it is manifestly impracticable to give even a summary of them here. As practiced by the Association of Official Agricultural Chemists, it is carried out as follows:¹²

(1) PREPARATION OF REAGENTS.—(a) *Iodine Solution*.—Dissolve 25 grams of pure iodine in 500 cubic centimeters of 95 per cent. alcohol. The latter solution, if necessary, is filtered and then the two solutions mixed. The mixed solution should be allowed to stand twelve hours before using.

(b) *Decinormal Sodium Thiosulfate Solution*.—Dissolve 24.8 grams of chemically pure sodium thiosulfate, freshly pulverized as finely as possible and dried between filter or blotting paper, and dilute with water to one liter, at the temperature at which the titrations are to be made.

(c) *Starch Paste*.—One gram of starch is boiled in 200 cubic centimeters of distilled water for 10 minutes and cooled to room temperature.

(d) *Solution of Potassium Iodide*.—One hundred and fifty grams of potassium iodide are dissolved in water and the volume made up to one liter.

(e) *Solution of Decinormal Potassium Bichromate*.—Dissolve 4.9066 grams of chemically pure potassium bichromate in distilled water and make the volume up to one liter at the temperature at which the titrations are to be made. The bichromate solution should be checked against pure iron.

(2) DETERMINATION.—(a) *Standardizing the Sodium Thiosulfate Solution*.—Place 20 cubic centimeters of the potassium

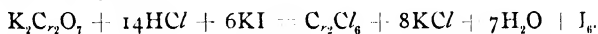
¹¹ Dinger's Polytechnisches Journal, 1884, 253 : 281.

Journal of the Society of Chemical Industry, 1884, 3 : 641.

¹² Division of Chemistry, Bulletin 46 (revised), 1899 : 50.

Bureau of Chemistry, Bulletin 107, 1912 : 136.

bichromate solution, to which have been added 10 cubic centimeters of the solution of potassium iodid, in a glass stopper flask. Add to this mixture five cubic centimeters of strong hydrochloric acid. Allow the solution of sodium thiosulfate to flow slowly into the flask until the yellow color of the liquid has almost disappeared. Add a few drops of the starch paste, and with constant shaking continue to add the sodium thiosulfate solution until the blue color just disappears. From the volume of thiosulfate used the equivalent of one cubic centimeter of thiosulfate in terms of iodine can be readily determined by the following equation:



Example:—Twenty cubic centimeters of potassium bichromate solution required 19.5 of the sodium thiosulfate. Since each cubic centimeter of decinormal bichromate is equivalent to 0.01269 grams of pure iodine, we have 19.5 cubic centimeters of the thiosulfate equivalent to 0.2538 grams or each cubic centimeter equal to 0.01301 gram of iodine.

(b) *Weighing the Sample.*—About a half gram of fat or one-fourth gram of oil is placed in a glass stopper flask, holding about 300 cubic centimeters, with the precautions to be mentioned for weighing the fat for determining volatile acids.

(c) *Absorption of Iodine.*—The fat in the flask is dissolved in 10 cubic centimeters of chloroform. After complete solution has taken place 30 cubic centimeters of the iodine solution (1) (a) are added. The flask is now placed in a dark place and allowed to stand, with occasional shaking, for three hours. The time allowed does not give the complete iodine absorption power of an oil or fat and can not be compared with determinations in which six to 12 hours have been used. It gives very satisfactory comparative results, but the time factor must be very closely observed.

(d) *Titration of the Unabsorbed Iodine.*—Twenty cubic centimeters of the potassium iodide solution together with 100 cubic centimeters of distilled water are added to the contents of the flask. Any iodine which may be noticed upon the stopper of the flask should be washed back into the flask with the potassium

iodid solution. The excess of iodine is taken up with the sodium thiosulfate solution, which is run in gradually, with constant shaking, until the yellow color of the solution has almost disappeared. A few drops of starch paste are added, and the titration continued until the blue color has entirely disappeared. Toward the end of the reaction the flask should be stoppered and violently shaken, so that any iodine remaining in solution in the chloroform may be taken up by the potassium iodide solution in the water. A sufficient quantity of sodium thiosulfate solution should be added to prevent a reappearance of any blue color in the flask for five minutes.

(c) *Setting the Value of the Iodine Solution by the Thiosulfate Solution.*—At the time of adding the iodine solution to the fat, two flasks of the same size as those used for the determination should be employed for conducting the operation described above, but without the presence of any fat. In every other respect the performance of the blank experiments should be just as described. These blank experiments must be made each time the iodine solution is used. Great care must be taken that there is no appreciable temperature change during the time of the operation. The proper correction for the blank is made.

Example of Iodine Determination.—Thirty cubic centimeters of iodine solution required 46.4 cubic centimeters of sodium thiosulfate solution: Thirty cubic centimeters of iodine solution required 46.8 cubic centimeters of sodium thiosulfate solution: Mean, 46.6 cubic centimeters.

Weight of fat.....	1.0479	grams
Quantity of iodine solution used.....	30.0	cubic centimeters
Thiosulfate equivalent to iodine used	46.6	" "
Thiosulfate equivalent to remaining iodine	14.7	" "
Thiosulfate equivalent to iodine absorbed	31.9	" "
Percent of iodine absorbed, $31.9 \times 0.01301 \times 100 \div 1.0479 = 39.61$.		

369. Character of Chemical Reaction.—The exact nature of the chemical process which takes place in this reaction is not definitely known. Hübl supposed that the products formed were chloro-

iodid-additive compounds, and he obtained a greasy product from oleic acid, to which he ascribed the formula $C_{18}H_{24}IClO_2$. By others it is thought that chlorin alone may be added to the molecule.¹³

In general, it may be said that none of the glycerids capable of absorbing halogens is able to take on a quantity equivalent to theory.¹⁴ While the saturated fatty acids (stearic series) theoretically are not able to absorb iodine some of them are found to do so to a small degree. It is evident, therefore, that it is not possible to calculate the percentage of unsaturated glycerids in a fat from their iodine number alone. According to the data worked out by Schweitzer and Lungwitz both addition and substitution of iodine take place during the reaction.¹⁵ This fact they determined by titration with potassium iodate and iodide according to the formula $5HI + IIO_3 = I + 6H_2O$. The authors confess that whenever free hydriodic acid is found in the mixture that iodine substitution has taken place and that for each atom of hydrogen eliminated from the fat molecule two atoms of iodine disappear, one as the substitute and the other in the form of hydriodic acid. When carbon bisulfide or tetrachloride is used as a solvent no substitution takes place and pure additive compounds are formed.

The following process is recommended to secure a pure iodine addition to a glycerid: About one gram or a little less of the oil or fat is placed in a glass stopper flask, to which are added about seven-tenths of a gram of powdered mercuric chloride and 25 cubic centimeters of a solution of iodine in carbon bisulfide. The stopper is made tight by smearing it with powdered potassium iodide, tied down, and the mixture is heated for some time under pressure. By this method it is found that no hydriodic acid is formed, and hence all the iodine which disappears is added to the

¹³ Liebermann, *Berichte der deutschen chemischen Gesellschaft*, 1891, **24**: 4117.

¹⁴ Lewkowitsch, *Chemical Technology and Analysis of Oils, Fats and Waxes*, 4th Edition, 1909.

¹⁵ *Journal of the Society of Chemical Industry*, 1895, : 130, 351 and 1030.

molecule of the glycerid. The additive numbers obtained for some oils are appended:

Oil	Time of heating	Temperature	Per cent iodine added	Per cent hyd number
Lard oil.....	30 minutes	50°.0	73.0	78.4
Cottonseed oil..	2 hours	50°.0	103.0	106.5
Oleic acid.....	2 "	65°.5	93.8

370. Solution in Carbon Tetrachlorid.—Gantter has called attention to the fact that the amount of iodine absorbed by fat does not depend alone upon the proportion of iodine present but also upon the amount of mercuric chlorid in the solution.¹⁶ Increasing amounts of mercuric chlorid cause uniformly a much greater absorption of the iodine. For this reason he proposes to discard the use of mercuric chlorid altogether for the above test and to use a solvent which will at the same time dissolve both the iodine and the fat. For this purpose he uses carbon tetrachlorid. The solutions are prepared as follows:

Iodine Solution.—Ten grams of iodine are dissolved in one liter of carbon tetrachlorid.

In the preparation of this solution the iodine must not be thrown directly into the flask before the addition of the tetrachlorid. Iodine dissolves very slowly in carbon tetrachlorid and the solution is made by placing it in a sufficiently large weighing glass and adding a portion of the carbon tetrachlorid thereto. The solution is facilitated by stirring with a glass rod until the added tetrachlorid is apparently charged with the dissolved iodine. The dissolved portion is then poured into a liter flask, new portions added to the iodine and this process continued until the iodine is completely dissolved, and then sufficient additional quantities of the tetrachlorid are added to fill the flask up to the mark.

371. Sodium Thiosulfate Solution.—Dissolve 19.528 grams of pure sodium thiosulfate in 1,000 cubic centimeters of water. For determining the strength of the solution by titration, the solution of iodine in carbon tetrachlorid and a solution of sodium thiosulfate in water are each placed in a burette. A given volume of the iodine solution is first run into a flask with a glass stopper and

¹⁶ Zeitschrift für analytische chemie, 1893, 32 : 181 et seq.

afterward the sodium thiosulfate added little by little until, after a vigorous shaking, the liquid has only a little color. Some solution of starch is then added and shaken until the mixture becomes deep blue. The sodium thiosulfate solution is added drop by drop, with vigorous shaking after each addition, until the solution is completely decolorized. If both solutions have been correctly made with pure materials they will be of equal strength; that is, 10 cubic centimeters of the iodine solution will be exactly decolorized by 10 cubic centimeters of the sodium thiosulfate solution.

372. Method of Conducting the Absorption.—The quantity of the fat or oil employed should range from 100 to 200 milligrams, according to the absorption equivalent. These quantities should be placed in flasks with glass stoppers in the ordinary way. In the flasks are placed exactly 50 cubic centimeters of the iodine solution equivalent to 500 milligrams of iodine, and the flask is then stoppered and shaken until the fat or oil is completely dissolved. In order to avoid the volatilization of the iodine finally, sufficient water is poured into the flask to form a layer about one millimeter in thickness over the solution containing the iodine and fat. The stopper should be carefully inserted and the flask allowed to stand at rest for 50 hours.

373. Estimation of the Iodine Number.—This is determined in the usual way by titration of the amount of iodine left in excess after the absorption as above described. The iodine number is to be expressed by the number of milligrams of iodine which are absorbed by each 100 milligrams of fat.

Example.—One hundred and one milligrams of flaxseed oil were dissolved in 50 cubic centimeters of the carbon tetrachloride solution of iodine and allowed to stand as above described for 50 hours. At the end of this time, 42.3 cubic centimeters of the sodium thiosulfate solution were required to decolorize the excess of iodine remaining.

Statement of Results.—Fifty cubic centimeters of the sodium thiosulfate equal 500 milligrams of iodine; therefore, 42.3 cubic centimeters of the thiosulfate solution equal 42.3 milligrams of

iodin. The difference equals 77 milligrams of iodine absorbed by 101 milligrams of the flaxseed oil. Therefore, the iodine number equivalent and the milligrams of iodine absorbed by 100 milligrams of flaxseed oil equal 76.2.

It is evident from the above determination that the iodine number of the oil, when obtained in the manner described, is less than half that secured by the usual Hübl process. Since the solvent employed, however, is more stable than chloroform when in contact with iodine or bromine, the proposed variation is one worthy of the careful attention of analysts.

McIlhenny has called especial attention to the low numbers given by the method of Gantter, and from a study of the data obtained concludes that iodine alone will not saturate glycerids, no matter what the solvents may be.¹⁷

It is clear, therefore, that the process of Gantter cannot give numbers which are comparable with those obtained by the usual iodine method. Any comparative value possessed by the data given by the process of Gantter must be derived by confining it to the numbers secured by the carbon tetrachloride process alone.

374. Substitution of Iodin Monochloride for Hübl's Reagent.--

Ephraim has shown that iodine monochloride may be conveniently substituted for the Hübl reagent with the advantage that it can be safely used at once, while the Hübl reagent undergoes somewhat rapid changes when first prepared. The present disadvantage of the process is found in the fact that the iodine monochloride of commerce is not quite pure and each new lot requires to be titrated for the determination of its purity.

The reagent is prepared of such a strength as to contain 16.25 grams of iodine monochloride per liter. The solvent used is alcohol. The operation is carried out precisely as in the Hübl method, substituting the alcoholic solution of iodine monochloride for the iodine reagent proposed by Hübl.¹⁸ If the iodine monochloride solution, after acting on the oil, be titrated without previous addition of potassium iodide a new value is obtained, the chloriodine number.

¹⁷ Journal of the American Chemical Society, 1894, 16 : 372.

The Analyst, 1895, 20 : 176.

¹⁸ Zeitschrift für angewandte Chemie, 1895 : 254.

In titrating, the sodium thiosulfate is added until the liquid, which is made brown by the separated iodine, becomes yellow. At this point the solution is diluted, starch paste added, and the titration completed.

375. Preservation of the Hübl Reagent.—To avoid the trouble due to changes in the strength of Hübl's reagent, Mahle adds hydrochloric acid to it at the time of its preparation.¹⁹ The reagent is prepared as follows: Twenty-five grams of iodine dissolved in a quarter of a liter of 95 per cent. alcohol are mixed with the same quantity of mercuric chlorid in 200 cubic centimeters of alcohol, the same weight of hydrochloric acid of 1.19 specific gravity added and the volume of the mixture completed to half a liter with alcohol. After five days such a solution gave, on titration, 49.18 instead of 49.31 grams per liter of iodine.

It will be observed that this solution is double the usual strength but this does not influence the accuracy of the analytical data obtained. It appears that the hübl number is not, therefore, an iodine number, but expresses the total quantity of iodine, chlorine and oxygen absorbed by the fat during the progress of the reaction.

375a. Hanus Method.—As a result of considerable discussion and cooperative work among the chemists of this country, interested in standard methods for oil analysis, the Association of Official Agricultural Chemists adopted the following procedure, usually known as the Hanus method, as official in 1904.^{19a}

Hanus Iodine Solution.—Dissolve 13.2 grams of iodine in a liter of glacial acetic acid, which shows no reduction with bichromate and sulfuric acid; then add enough bromine to double the halogen content as determined by titration. It will be found advantageous to grind the iodine as fine as possible in a mortar and if necessary the acetic acid may be warmed a little to aid the solution of the iodine but must be cool before the bromine is added.

The thiosulfate, starch and potassium iodide are made up as

¹⁹ Chemiker-Zeitung, , 19 : 1786, 1831.

^{19a} Bureau of Chemistry, Bulletin 81, 1904 : 46-65.

Zeitschrift für Untersuchung der Nahrungs und Genussmittel, 1901.

in the official hübl method and the same amount of sample as indicated is used. Only 25 cubic centimeters of the halogen solution need be added to the chloroform solution of the fat and an hour is sufficient time to allow the mixture to stand. Dilution and titration of excess of iodine, which should be at least 60 per cent., are carried out as before except only 10 cubic centimeters of potassium iodide need be used to hold the free iodine in solution during final titration.

Wijs²⁰ has developed a method very similar to that of Hanus in which chlorine is used as the second haloid instead of bromine. The figures obtained by his procedure are nearly always higher than by either the hanus or hübl process. As a great deal of valuable data have already been published, based upon the older hübl method and the keeping qualities of the hanus solution is equally as good and the operation as expeditious as with Wijs, the former, which gives figures only slightly different from those obtained by Hübl, is to be preferred. The comparative results of the three processes determined by Tolman and Munson and their collaborators show in the main satisfactory agreement.²¹

The hübl method, especially on the oils with high iodine numbers, shows many discrepancies between the results of the various analysts. The Wijs method gave the best results; especially is this true with linseed oil where the maximum difference between the results of the five analysts was 2.00 numbers. The hanus method also gives good results, which are uniformly lower than those of the Wijs method, making a closer agreement with figures at present obtained by the hübl method. This agreement, however, is not of great value on these oils, especially the rape, mustard, maize, poppy, and linseed oils, as the published data give such wide limits.

These wide limits are doubtless largely due to the varying methods and modifications of the hübl method used in the determination. The hübl solutions used in this work were not more than 24 hours old, and the results show this wide variation.

²⁰ *Berichte der Deutschen chemischen Gesellschaft*, 1898, **31** : 750;
Journal Society Chemical Industry, 1902, **21** : 455.

²¹ *Bureau of Chemistry, Bulletin* 81 : 51.

If they had varied in age from one week to three weeks, probably a very much wider variation would have been found.

376. Bromin Addition Number.—In the process of Hübl and others an attempt is made to determine the quantity of a halogen, *e. g.*, iodine, which the oil, fat or resin will absorb under certain conditions. The numbers obtained, however, represent this absorption only approximately, because the halogen may disappear through substitution as well as absorption. Whether or not a halogen is added, *i. e.*, absorbed or substituted, may be determined experimentally.

The principle on which the determination depends rests on the fact that a halogen, *e. g.*, bromine, forms a molecule of hydrobromic acid for every atom of bromine substituted, while in a simple absorption of the halogen no such action takes place. If, therefore, bromine be brought into contact with a fat, oil or resin, the determination of the quantity of hydrobromic acid formed will rigidly determine the quantity of bromine substituted during the reaction. If this quantity be deducted from the total bromine which has disappeared, the relative quantities of the halogen added and substituted are at once determined. In the method of McIlhenny²² bromine is used instead of iodine because the addition figures of iodine are in general much too low.

The Reagents.—The following solutions are employed:

1. One-third normal bromine dissolved in carbon tetrachloride.
2. One-tenth normal sodium thiosulfate.
3. One-tenth normal potassium hydroxide.

The Manipulation.—From a quarter to one gram of the fat, oil or resin, is dissolved in 10 cubic centimeters of carbon tetrachloride in a dry bottle of 500 cubic centimeters capacity, provided with a well-ground glass stopper. An excess of the bromine solution is added, the bottle tightly stoppered, well shaken and placed in the dark. At the end of 18 hours the bottle is placed in a freezing mixture and cooled until a partial vacuum is formed. A piece of wide rubber tubing an inch and a half long is slipped

²² Journal of the American Chemical Society, 1894, 16 : 277.

Journal Society Chemical Industry, 1894, 13 : 668.

over the lip of the bottle so as to form a well about the stopper. This well having been filled with water the stopper is lifted and the water is sucked into the bottle absorbing all the hydrobromic acid which has been formed. The well should be kept filled with water, as it is gradually taken in until in all 25 cubic centimeters have been added. The bottle is next well shaken and from 10 to 20 cubic centimeters of a 20 per cent. potassium iodid solution added.

The excess of bromin liberates a corresponding amount of iodine, which is determined by the thiosulfate solution in the usual way, after adding about 75 cubic centimeters of water. The total bromin which has disappeared is then calculated from the data obtained, the strength of the original bromin solution having been previously determined. The contents of the bottle are next transferred to a separatory funnel, the aqueous portion separated, filtered through a linen filter, a few drops of thiosulfate solution added, if a blue color persist, and the free hydrobromic acid determined by titration with potassium hydroxid, using methyl orange as indicator. The end reaction is best observed by placing the solution in a porcelain dish, adding the alkali in slight excess, and titrating back with tenth-normal hydrochloric acid until the pink tint is perceived. From the number of cubic centimeters of alkali used the amount of bromin present as hydrobromic acid is calculated, and this expressed as percentage gives the bromin substitution figure. The bromin substitution figure multiplied by two and subtracted from the total absorption gives the additional figure.

Following are the data for some common substances:

Substance	Total bromin absorption in eighteen hours	Bromin addi- tion figure	Bromin substi- tution figure
Rosin	212.70	0.00	106.35
Raw linseed oil	102.88	102.88	00.00
Boiled " "	103.92	103.92	00.00
Salad cotton "	65.54	64.26	0.64
Sperm "	56.60	54.52	1.04

By the process just described it is possible to detect mixtures

of rosins and rosin oils with animal and vegetable oils. In this respect it possesses undoubted advantages over the older methods.

377. Method of Hehner.—The absorption of bromin which takes place when unsaturated fats are brought into contact with that reagent was made the basis of an analytical process, proposed by Allen as long ago as 1880.²³ In the further study of the phenomena of bromin absorption, as indicated by McIlhiney, Hehner modified the method as indicated below.²⁴ From one to three grams of the sample are placed in a tared wide-mouthed flask and dissolved in a little chloroform. Bromin is added to the solution, drop by drop, until it is in decided excess. The flask is placed on a steam-bath and heated until the greater part of the bromin is evaporated, when some more chloroform is added and the heating continued until all the free bromin has escaped. The flask is put in a bath at 125° and dried to constant weight. A little acrolein and hydrobromic acid escape during the drying and the residue may be colored, or a heavy bromo oil be obtained. The gain in weight represents the bromin absorbed. The bromin number may be converted into the iodine number by multiplying by 1.5875.²⁵ The more recent work on this method by Lewkowitsch,²⁶ Jenkins²⁷ and Proctor²⁸ indicates that this procedure is not of general applicability, olive and a few similar oils yield satisfactory results but in many cases great discrepancies between figures obtained by this method and Hübl's were observed.

378. Halogen Absorption and Addition of Fat Acids.—Instead of employing the natural glycerids for determining the degree of action with the halogens the acids may be separated by some of the processes of saponification hereafter described and used as directed for the glycerids themselves. It is doubtful if any practical advantage arises from this variation of the

²³ Pharmaceutical Journal, 1880, September 25.

²⁴ The Analyst, 1895, 20 : 50, 148.

²⁵ Williams, The Analyst, 1895, 20 : 277.

²⁶ Journal Society of Chemical Industry, 1896, 15 : 859.

²⁷ Journal Society of Chemical Industry, 1897, 16 : 193.

²⁸ Journal Society of Chemical Industry, 1906, 25 : 799.

process. If the fat acids be separated, however, it is possible to get some valuable data from the halogen absorption of the fractions. Theoretically the stearic series of acids would suffer no change in contact with halogens while the oleic series is capable of a maximum absorptive and additive action. On this fact is based a variation of the iodine process in which an attempt is made to separate the oleic acid from its congeners and to apply the halogen to the separated product.

The method of separation devised by Muter is carried out as follows:²⁹ The separatory or olein tube consists of a wide

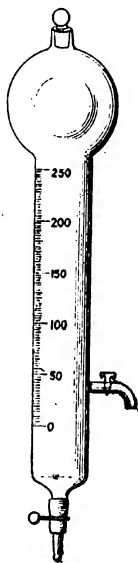


Fig. 93.—Olein Tube.

burette stem, provided with a lateral stop-cock, and drawn out below to secure a clamp delivery tube, and at the top expanded into a bulb closed with a ground glass stopper, as shown in Fig. 93. Forty cubic centimeters of liquid are placed in the tube and the surface is marked 0. Above this the graduation is continued

²⁹ *The Analyst*, 1889, 14 : 61.

in cubic centimeters to 250, which figure is just below the bulb at the top.

The process of analysis is conducted as follows: About three grams of the oil or fat are placed in a flask, with 50 cubic centimeters of alcoholic potash lye, containing enough potassium hydroxid to ensure complete saponification. The flask is closed and heated on a water-bath until saponification is complete. The pressure flask to be described hereafter may be conveniently used. After cooling, the excess of alkali is neutralized with acetic acid in presence of phenolphthalein and then alcoholic potash added until a faint pink color is produced. In a large porcelain dish place 200 cubic centimeters of water and 30 of a 10 per cent. solution of lead acetate and boil. Pour slowly, with constant stirring, into the boiling liquid the soap solution prepared as above described, and allow to cool, meanwhile continuing the stirring. At the end, the liquid remaining is poured off and the solid residue washed with hot water by decantation.

The precipitate of lead salts is finally removed from the dish into a stoppered bottle, the dish washed with pure ether, the washings added to the bottle together with enough ether to make the total volume thereof 120 cubic centimeters. The closed bottle is allowed to stand for 12 hours with occasional shaking, by which time the lead oleate will have been completely dissolved. The insoluble lead salts are next separated by filtration, and the filtrate collected in the olein tube. The washing is accomplished by ether and, to avoid loss, the funnel is covered with a glass plate. The ethereal solution of lead oleate is decomposed by dilute hydrochloric acid, using about 40 cubic centimeters of a mixture containing one part of strong acid to four of water. The olein tube is closed and shaken until the decomposition is complete, which will be indicated by the clearing of the ethereal solution. The tube is allowed to remain at rest until the liquids separate and the aqueous solution is run out from the pinch-cock at the lower end. The residue is washed with water by shaking, the water drawn off as just described, and the process continued until all acidity is removed.

Water is then added until the separating plane between the two liquids is at the zero of the graduation, and enough ether added to make the ethereal solution of a desired volume, say 200 cubic centimeters. After well mixing, the ethereal solution or an aliquot part thereof, *e. g.*, 50 cubic centimeters, is removed by the side tubulure and nearly the whole of the ether removed from the portion by distillation. To the residue are added 50 cubic centimeters of pure alcohol and the solution is titrated for oleic acid with decinormal sodium hydroxid solution. Each cubic centimeter of the hydroxid solution used is equivalent to 0.0282 gram of oleic acid. The total quantity of oleic acid contained in the amount of fat used is readily calculated from the data obtained.

To determine the iodine absorption of the free acid another measured quantity of the ethereal solution containing as nearly as possible half a gram of oleic acid, is withdrawn from the olein tube, and the ether removed in an atmosphere of pure carbon dioxide. To the residue, with allowing it to come in contact with the air, 50 cubic centimeters of Hübl's reagent are added and the flask put aside in the dark for 12 hours. At the end of this time 35 cubic centimeters of a 10 per cent. solution of potassium iodide are added, the contents of the flask made up to a quarter of a liter with water, 15 cubic centimeters of chloroform added, and the excess of iodine titrated in the way already described. The percentage of iodine absorbed is calculated as already indicated.

Lane has proposed a more rapid process for the above determination.³⁰ The lead soaps are precipitated in a large Erlenmeyer and cooled rapidly in water, giving the flask meanwhile a circular motion which causes the soaps to adhere to its walls. Wash with hot water, rinsing once with alcohol, add 120 cubic centimeters of ether, attach a reflux condenser, and boil until the lead oleate is dissolved, cool slowly, to allow any lead stearate which has passed into solution to separate, and filter into the olein tube. The rest of the operation is conducted as described above.

³⁰ Journal of the American Chemical Society, 1893, **15** : 110.

The percentage of oleic acid and its iodine absorption in the following glycerids are given in the table below:

	Cottonseed oil	Lard	Peanut oil
Per cent oleic acid	75.16	64.15	79.84
Per cent iodine absorbed . . .	141.96	99.48	114.00

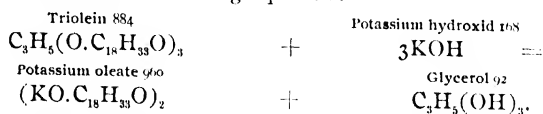
379. Saponification.—In many of the analytical operations which are conducted on the glycerids it is necessary to decompose them. When this is accomplished by the action of a base which displaces the glycerol from its combination with the fat acids, the resulting salts are known as soaps and the process is named saponification. In general use the term saponification is applied, not only strictly, as above defined, but also broadly, including the setting free of the glycerol either by the action of strong acids or by the application of superheated steam. In chemical processes the saponification of a glycerid is almost always accomplished by means of caustic soda or potash. This may be in aqueous or alcoholic solution and the process is accomplished either hot or cold, in open vessels or under pressure. It is only important that the alkali and glycerid be brought into intimate contact. The rate of saponification is a function of the intimacy of contact, the nature of the solvent and the temperature. For chemical purposes, it is best that the decomposition of the glycerid be accomplished at a low temperature and for most samples this is secured by dissolving the alkali in alcohol.

In respect of solvents, that one would be most desirable, from theoretical considerations, which acts on both the glycerids and alkalis. In the next rank would be those which dissolve one or the other of the materials and are easily miscible, as, for instance, carbon tetrachloride for the glycerid and alcohol for the alkali. As a rule, the glycerid is not brought into solution before the saponification process is commenced. Instead of using an alcoholic solution of sodium or potassium hydroxide the sodium or potassium alcoholate may be employed, made by dissolving metallic sodium or potassium in alcohol. It is probable, however, that a little water is always necessary to complete the process.

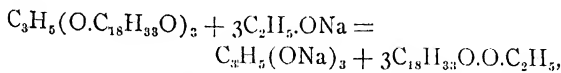
If a fat be dissolved in ether and treated with sodium alcoholate, a granular deposit of soap is soon formed and the saponifica-

tion is completed in 24 hours. As much as 150 grams of fat can be saponified with 10 grams of metallic sodium dissolved in 250 cubic centimeters of absolute alcohol.³¹ For practical purposes the alcoholic solution of the hydroxid is sufficient.

The chemical changes which fats undergo on saponification are of a simple kind. When the process is accomplished by means of alkalis, the alkaline base takes the place of the glycerol as indicated in the following equation:



The actual changes which take place in ordinary saponification are not so simple, however, since natural glycerids are mixtures of several widely differing fatty acids and glycerol, each of which has its own rate of decomposition. Palmitin and stearin, for instance, are saponified more readily than olein and some of the saponifiable constituents of resins and waxes are extremely resistant to the action of alkalis. The above equation may be regarded as typical for saponification in aqueous or alcoholic solutions in open dishes or under pressure. If the alkali used be prepared by dissolving metallic sodium or potassium in absolute alcohol (sodium alcoholate or ethoxid) the reaction which takes place is probably represented by the equation given below:



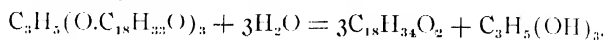
in which it is seen that complete saponification cannot occur without the absorption of some water, by which the sodium glyceroxid is converted into glycerol and sodium hydroxid, the latter compound eventually uniting with the ethyl ether of the fat acid.³²

Glycerids are decomposed when heated with water under a

³¹ Zeitschrift für physiologische Chemie, 14 : 599; 12 : 321; 16 : 152.

³² Lewkowitsch, Chemical Technology and Analysis of Oils, Fats, Waxes, 4th Edition, 1909 : Vol. I, 79.

pressure of about 16 atmospheres or when subjected to a current of superheated steam at 200°. The reaction consists in the addition of the elements of water, whereby the glyceryl radicle is converted into free glycerol and the fat acid is set free. The chemical change which ensues is shown below:



The details of saponification with sulfuric acid are of no interest from an analytical point of view.³³

380. Saponification in an Open Dish.—The simplest method of saponifying fats is to treat them with the alkaline reagent in an open dish. In all cases the process is accelerated by the application of heat. Vigorous stirring also aids the process by securing a more intimate mixture of the ingredients. This method of decomposing glycerids, however, is not applicable in cases where volatile ethers may be developed. These ethers may escape saponification and thus prevent the formation of the maximum quantity of soap. While not suited to exact quantitative work, the method is convenient in the preparation of fat acids which are to be the basis of subsequent analytical operations, as, for instance, in the preparation of fat acids for testing with silver nitrate or the titre test. Large porcelain dishes are conveniently used and the heat is applied in any usual way, with care to avoid scorching the fat.

381. Saponification under Pressure.—The method of saponification which has been adopted by the Association of Official Agricultural Chemists is described below.³⁴

Reagents.—The reagents employed are a solution of pure potash containing 100 grams of the hydroxid dissolved in 58 grams of recently boiled distilled water, alcohol of approximately 95 per cent. strength redistilled over caustic soda, and sodium hydroxid solution prepared as follows:

One hundred grams of sodium hydroxid are dissolved in 100 cubic centimeters of distilled water. The caustic soda should

³³ Lewkowitsch, *Chemical Technology and Analysis of Oils, Fats, Waxes*, 4th Edition, 1909: Vol. I, 79.

³⁴ Division of Chemistry, Bulletin 13, part 4, 1889: 459; Bulletin 46 (revised), 1899: 44; Bureau of Chemistry, Bulletin 107 (revised), 1912: 137.

be as free as possible from carbonates, and be preserved from contact with the air.

Apparatus.—A saponification flask is used which has a round bottom and a ring near the top, by means of which the stopper can be tied down. The flask is arranged for heating as shown in Fig. 94. A pipette graduated to deliver 40 cubic centimeters

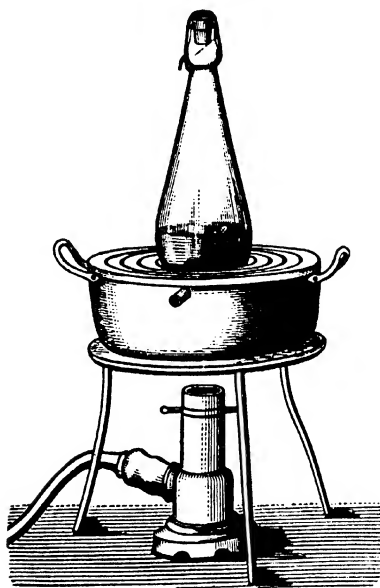


Fig. 94. Apparatus for Saponifying under Pressure.

is recommended as being more convenient than a burette for measuring the solutions. A pipette with a long stem graduated to deliver 5.75 cubic centimeters at 50° is also required.

Manipulation.—The fat to be examined should be melted and kept in a dry warm place at about 60° for two or three hours, until the water has entirely separated. The clear supernatant fat is poured off and filtered through a dry filter paper in a jacket funnel containing boiling water. Should the filtered fat, in a

fused state, not be perfectly clear, it must be filtered a second time. The final drying is accomplished at 100° in a thin layer in a flat bottom dish, in partial vacuum or an atmosphere of inert gas.

The saponification flasks are prepared by thoroughly washing with water, alcohol, and ether, wiping perfectly dry on the outside, and heating for one hour at the temperature of boiling water. The hard flasks used in moist combustions with sulfuric acid for the determination of nitrogen are well suited for this work. The flasks should be placed in a tray by the side of the balance and covered with a cloth until they are perfectly cool. They must not be wiped with a silk handkerchief within 15 or 20 minutes of the time they are weighed or else the electricity developed will interfere with weighing. The weight of the flasks having been accurately determined, they are charged with the melted fat in the following way:

The pipette with a long stem, marked to deliver 5.75 cubic centimeters, is warmed to a temperature of about 50° . The fat, having been poured back and forth once or twice into a dry beaker in order to thoroughly mix it, is taken up in the pipette, the nozzle of the pipette having been previously wiped to remove any externally adhering fat, is carried to near the bottom of the flask and 5.75 cubic centimeters of fat allowed to flow into the flask. After the flasks have been charged in this way they should be recovered with the cloth and allowed to stand for 15 or 20 minutes, when they are again weighed.

382. Methods of Saponification.—*In the Presence of Alcohol.*—Ten cubic centimeters of 95 per cent. alcohol are added to the fat in the flask, and then two cubic centimeters of the sodium hydroxid solution. A soft cork stopper is inserted and tied down with a piece of twine. The saponification is completed by placing the flask upon the water or steam-bath. The flask during the saponification, which should last one hour, should be gently rotated from time to time, being careful not to project the soap for any distance up its sides. At the end of an hour the flask, after having been cooled to near the room temperature, is opened.

Without the Use of Alcohol.—To avoid the danger of loss from

the formation of ethers, and the trouble of removing the alcohol after saponification, the fat may be saponified with a solution of caustic potash in a closed flask without using alcohol. The operation is carried on exactly as indicated above for saponification in the presence of alcohol, using potassium instead of sodium hydroxid solution. For the saponification, use two cubic centimeters of the potassium hydroxid solution which are poured on the fat after it has solidified in the flask. Great care must be taken that none of the fat be allowed to rise on the sides of the saponifying flask to a point where it cannot be reached by the alkali. During the process of saponification the flask can only be very gently rotated in order to avoid the difficulty mentioned. This process is not recommended with any apparatus except a closed flask with round bottom. Potash is used instead of soda so as to form a softer soap and thus allow a more perfect saponification.

With a Reflux Condenser and the Use of Alcohol.—Place 10 cubic centimeters of the 95 per cent. alcohol in the flask containing the fat, add two cubic centimeters of the sodium hydroxid solution with a reflux condenser (a glass tube not less than one meter in length is allowable), and heat on the steam-bath until the saponification is complete.

383. Saponification in the Cold.—By reason of the danger of loss from volatile ethers in the hot alcoholic saponification, a method for successfully conducting the operation in the cold is desirable. Such a process has been worked out by Henriques.³⁵ It is based upon the previous solution of the fat in petroleum ether, in which condition it is so easily attacked by the alcoholic alkali as to make the use of heat during the saponification unnecessary. The process is conveniently conducted in a porcelain dish covered with a watch glass. Five grams of the fat are dissolved in 25 cubic centimeters of petroleum ether and treated with an equal quantity of four per cent. alcoholic soda lye. The process of saponification begins at once and is often indicated by the separation of sodium salts. It is best to allow the action to continue over night and, with certain difficultly saponifiable

³⁵ *Zeitschrift für angewandte Chemie*, 1895 : 721.

bodies, such as wool fat and waxes, for 24 hours. In the case of butter fat an odor of butyric ether may be perceived at first but it soon disappears. After the saponification is complete, the excess of alkali is determined by titration in the usual way with set hydrochloric acid, using phenolphthalein as indicator. For the determination of volatile acids, the mixture, after saponification is complete, dried at a low temperature, the solid matter being reduced to powder with a glass rod, after which it is transferred to a distilling flask and the volatile acids secured by the usual processes. In comparison with the saponification and Reichert-Meissl numbers obtained with hot alcoholic potash, the numbers given by the cold process are found to be slightly higher with those fats which give easily volatile ethers. On account of the simplicity of the process and the absence of danger of loss from ethers, it is to be recommended instead of the older methods in case a more extended trial of it should establish the points of excellence claimed above.

384. Saponification Value.—The number of milligrams of potassium hydroxid required to completely saturate one gram of a fat is known as the saponification value of the glycerid. The process of determining this value, as worked out by Koettstorfer and modified in the laboratory of the Department of Agriculture, and adopted by the Association of Official Agricultural Chemists, is as follows:³⁶

REAGENTS.—(1) *Standard Sodium Hydroxid.*—Use a tenth-normal solution each cubic centimeter of which contains 0.0040 gram of sodium hydroxid and will neutralize 0.0088 gram of butyric acid.

(2) *Alcoholic Potash Solution.*—Dissolve 40 grams of chemically pure potassium hydroxid in one liter of 95 per cent. redistilled alcohol. The solution must be clear and the potassium hydroxid free from carbonates.

(3) *Standard Acid Solution.*—Prepare accurately a half-normal solution of hydrochloric acid.

³⁶ Zeitschrift für analytische Chemie, 1879, 18 : 199; Division of Chemistry, Bulletin 13, part 1, 1887 : 58, part 4, 1889 : 461; Bulletin 46, revised, 1899 : 48; Bulletin 107 (revised), 1912 : 137.

(4) *Indicator*.—Dissolve one gram of phenolphthalein in 100 cubic centimeters of 95 per cent. alcohol.

Determination.—Conduct the saponification in a wide-mouth Erlenmeyer flask holding from 250 to 300 cubic centimeters. Clean thoroughly by washing with water, alcohol, and ether, wipe perfectly dry on the outside and heat for one hour at the temperature of boiling water; allow to cool and weigh.

Run in about five grams of the filtered melted fat by means of a pipette, and after cooling again weigh the flask and contents. Pipette 50 cubic centimeters of the alcoholic potash solution into a flask by allowing it to drain for a definite time. Connect the flask with a reflux condenser and boil for 30 minutes or until the fat is completely saponified. Cool and titrate with half-normal hydrochloric acid using phenolphthalein as indicator. The Koettstorfer number (milligrams of potassium hydroxid required to saponify one gram of fat) is obtained as follows: Subtract the number of cubic centimeters of hydrochloric acid used to neutralize the excess of alkali after saponification from the number of cubic centimeters necessary to neutralize the 50 cubic centimeters of alkali added; multiply the result by 28.06 (the number of milligrams of potassium hydroxid per cubic centimeter) and divide by the number of grams of fat used. Great care must be exercised in measuring the alkaline solution, the same pipette being used in each case and the same time for draining being allowed in every instance. Blanks are always to be conducted with each series of examinations. As soon as the saponification is complete, the flask is removed from the bath, allowed to cool and its contents are titrated with semi-normal hydrochloric acid and phenolphthalein as indicator. The number expressing the saponification value is obtained by subtracting the number of cubic centimeters of semi-normal hydrochloric acid required to neutralize the alkali after saponification from that required to neutralize the alkali of the blank determinations, multiplying the result by 28.06 and dividing the product by the number of grams of fat employed.

Example.—Weight of sample of fat used 1.532 grams; Number of cubic centimeters half-normal hydrochloric acid required

to saturate blank, 22.5; Number of cubic centimeters of half-normal hydrochloric acid required to saturate the alkali after saponification 12.0; Difference, 10.5 cubic centimeters. Then

$$10.50 \times 28.06 \div 1.532 = 192.3.$$

This latter number represents the saponification value of the sample.

In the case of some of the waxes, as bees and wool wax, the saponification can only be completed by boiling over a flame for an hour or more. The resulting soap solution in the case of heavy oils and crude products is often so dark as to obscure considerably the end point, and alkaline blue as recommended by De Wagei and Fabris, or O. Schütte,³⁷ may advantageously be substituted for the phenolphthalein.

385. Saponification Equivalent.—Allen defines the saponification equivalent as the number of grams of fat saponified by one equivalent, *viz.*, 56.1 grams of potassium hydroxid.³⁸ The saponification equivalent is readily calculated from the saponification value using it as a divisor and 56100 as a dividend. Conversely the saponification value may be obtained by dividing 56100 by the saponification equivalent. No advantage is gained by the introduction of a new term so nearly related to saponification value.

386. Ester Value.—By the term ester value is meant the amount of alkali necessary to saponify the ester present and is found by subtracting the milligrams of KOH required to neutralize the free fatty acid in one gram of fat from the saponification value similarly expressed. As there is only a difference of five per cent. between the amount of KOH required to saponify 100 parts of stearin and to neutralize 100 parts of stearic acid, the change due to varying degrees of rancidity in a fat has much less effect upon the saponification value than on the ester number and the former only can be looked upon as a constant. 'This is very plainly shown by the following table:³⁹

³⁷ Chemiker Zeitung, **34** : 351.

³⁸ Bureau of Chemistry, Bulletin 107, revised, 1912 : 137.

Allen, Commercial Organic Analysis, 1899, **2** : 53, 56.

³⁹ Lewkowitsch, Chemical Technology and Analysis of Fats, Oils and Waxes, 4th Edition, 1909, **1** : 301.

COMPARISON OF SAPONIFICATION VALUE, ESTER
NUMBER AND ACID NUMBER

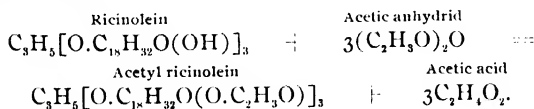
Per cent. stearin	Per cent. stearic acid	Saponification value	Acid number	Ester number
100	0	189.1	0.0	189.1
75	25	191.0 ²	49.37	141.65
50	50	193.3 ⁰	98.75	94.55
25	75	195.41	148.13	47.28
0	100	197.5	197.5	0.0

387. Saponification Value of Pure Glycerids.—The theoretical saponification values of pure glycerids are given in the following table:⁴⁰

Name	Symbol	Molecular weight	Saponification value
Butyrin	$C_3H_5(O.C_4H_7O)_3$	302	557.3
Valerin	$C_3H_5(O.C_5H_9O)_3$	344	489.2
Caproin	$C_3H_5(O.C_6H_{11}O)_3$	386	436.1
Caprin	$C_3H_5(O.C_{10}H_{19}O)_3$	554	303.7
Laurin	$C_3H_5(O.C_{12}H_{23}O)_3$	638	263.8
Myristin	$C_3H_5(O.C_{14}H_{27}O)_3$	722	233.1
Palmitin	$C_3H_5(O.C_{16}H_{31}O)_3$	806	208.8
Stearin	$C_3H_5(O.C_{18}H_{35}O)_3$	890	189.1
Olein	$C_3H_5(O.C_{18}H_{33}O)_3$	881	190.4
Linolein	$C_3H_5(O.C_{18}H_{31}O)_3$	878	191.7
Ricinolein	$C_3H_5(O.C_{18}H_{33}O_2)_3$	932	180.6
Euricin	$C_3H_5(O.C_{22}H_{41}O)_3$	1052	160.0

From the above table it is seen that in each series of glycerids the saponification equivalent falls as the molecular weight rises.

388. Acetyl Value.—Hydroxy acids and alcohols, when heated with glacial acetic acid, undergo a change which consists in substituting the radicle of acetic acid for the hydrogen atom of the alcoholic hydroxyl group. This change is illustrated by the equations below:⁴¹



For an Alcohol :



⁴⁰ Lewkowitsch, Chemical Technology and Analysis of Oils, Fats and Waxes, 4th Edition, 1909 ; Vol. 1, 399.

⁴¹ Lewkowitsch, Chemical Technology and Analysis of Oils, Fats and Waxes, 4th Edition, 1909, 1 : 337, 339.

Determination.—The method of determining the acetyl value of a fat or alcohol was first described by Benedikt and Ulzer,⁴² who conducted the operation on the fat acids and not on the glycerids containing them.

The insoluble fat acids are prepared as directed in paragraph 391.

From 20 to 50 grams of the fat acids are boiled with an equal volume of acetic anhydrid, in a flask with a reflux condenser, for two hours. The contents of the flask are transferred to a larger vessel of about one liter capacity, mixed with half a liter of water and boiled for half an hour. To prevent bumping, some bubbles of carbon dioxid are drawn through the liquid by means of a tube drawn out to a fine point and extending nearly to the bottom of the flask. The liquids are allowed to separate into two layers and the water is removed with a syphon. The oily matters are treated several times with boiling water until the acetic acid is all washed out. The acetylated fat acids are filtered through a dry hot jacket filter and an aliquot part, from three to five grams, is dissolved in absolute alcohol. After the addition of phenolphthalein the mixture is titrated as in the determination of the saponification value. The acid value thus obtained is designated as the acetyl acid value. A measured quantity of alcoholic potash, standardized by semi-normal hydrochloric acid, is added, the mixture boiled and the excess of alkali determined by titration. The quantity of alkali consumed in this process measures the acetyl value. The sum of the acetyl acid and the acetyl values is the acetyl saponification value. The acetyl value is therefore equal to the difference of the saponification and acid values of the acetylated fat acids. In other words, the acetyl value indicates the number of milligrams of potassium hydroxid required to neutralize the acetic acid obtained by the saponification of one gram of the acetylated fat acids.

Example.—A portion of the fat acids acetylated as described, weighing 3.379 grams, is exactly neutralized by 17.2 cubic centimeters of semi-normal potassium hydroxid solution, correspond-

⁴² Monatshefte für Chemie und verwandte Theile Anderer Wissenschaften, 8 : 40.

ing to $17.2 \times 0.02805 = 0.4825$ gram of the hydroxid, hence $0.4825 \times 1000 \div 3.379 = 142.8$, the acetyl acid value of the sample.

After the addition of 32.8 cubic centimeters more of the semi-normal potash solution, the mixture is boiled to saponify the acetylated fat acids. The residual potash requires 14.2 cubic centimeters of semi-normal hydrochloric acid. The quantity of potash required for the acetic acid is therefore $32.8 - 14.3 = 18.5$ cubic centimeters or $18.5 \times 0.02805 = 0.5189$ gram of potassium hydroxid. Then $0.5189 \times 1000 \div 3.379 = 153.6 =$ acetyl value of sample. The sum of these two values, *viz.*, 142.8 and 153.6 is 296.4, which is the acetyl saponification value of the sample. As with the iodine numbers, however, it is also found that acids of the oleic series give an acetyl value when treated as above, and it has been proposed by Lewkowitsch to determine, in lieu of the data obtained, the actual quantity of acetic acid absorbed by fats.⁴³

Benedikt-Lewkowitsch Method.— Boil the oil or fat with an equal volume of acetic anhydrid for two hours, pour the mixture into a large beaker containing 500 cubic centimeters of water, and boil for half an hour. To prevent bumping, pass a slow current of carbonic acid into the liquid through a finely drawn out tube reaching nearly to the bottom. Allow the mixture to separate into two layers, siphon off the water, and boil the oily layer with fresh water until it is no longer acid to litmus paper.

Separate the acetylated fat from the water and dry and filter in a drying oven.

Weigh from two to four grams of the acetylated fats into a flask and saponify with alcoholic potash as in the determination of saponification equivalent. If the distillation process is to be adopted it is not necessary to work with a standardized alcoholic potash solution, but in the filtration method, which is much shorter, the alcoholic potash must be measured exactly. In either

⁴³ Journal of the Society of Chemical Industry, 1897, 16 : 503.

Benedikt, *Analyse der Fette und Wachsarten*, 3d Edition, 1897 : 146; Allen, *Commercial Organic Analysis*, 3d Edition, 1899, 2 (1) : 66.

case evaporate the alcohol after saponification and dissolve the soap in water. Then either distil or filter as follows:

(a) *Distillation*.—Acidify with dilute sulfuric acid (1 to 10) and distil as in the Reichert-Meissl test. As several hundred cubic centimeters must be distilled, either run a current of steam through or add portions of water from time to time. From 500 to 700 cubic centimeters of distillate will be sufficient. Filter the distillates to remove any insoluble acids carried over by the steam, and titrate with tenth-normal potassium hydroxid, using phenolphthalein as indicator. Multiply the number of cubic centimeters of alkali employed by 5.61 and divide by the weight of substance used to obtain the acetyl value.

(b) *Filtration*.—Add to the soap solution a quantity of standard sulfuric acid exactly corresponding to the amount of alcoholic potash added, warm gently, filter off the free fatty acids which collect on top, wash with boiling water until the washings are no longer acid, and titrate the filtrate with tenth-normal potassium hydroxid, using phenolphthalein as indicator. Calculate the acetyl value as directed under (a).

The rôle which the acetyl value plays in analytical determinations is interesting, but the data it gives are not to be valued too highly.

389. Determination of Volatile Fat Acids.—The fat acids which are volatile at the temperature of boiling water, consist chiefly of butyric and its associated acids occurring in the secretions of the mammary glands. Among vegetable glycerids cocoanut oil is the only common one which has any notable content of volatile acids. The boiling points of the above acids, in a pure state, are much higher than the temperature of boiling water; for instance, butyric acid boils at about 162° . By the expression volatile acids, in analytical practice, is meant those which are carried over at 100° , or a little above, with the water vapor, whatever be their boiling point. The great difficulty of removing the volatile from the non-volatile fat acids has prevented the formulation of any method whereby a sharp and complete separation can be accomplished. The analyst, at the present time, must be content with some approximate process which, under like conditions, will

give comparable results. Instead, therefore, of attempting a definite determination, he confines his work to securing a partial separation and in expressing the degree of volatile acidity in terms of a standard alkali. To this end, a definite weight of the fat is saponified, the resulting soap decomposed with an excess of fixed acid, and a definite volume of distillate collected and its acidity determined by titration with decinormal alkali. The weight of fat operated on is either two and a half⁴⁴ or five grams.⁴⁵

Numerous minor variations have been proposed in the process, the most important of which is in the use of phosphoric instead of sulfuric acid in the distillation. An extended experience with both acids has shown that no danger is to be apprehended in the use of sulfuric acid and that on the whole it is to be preferred to phosphoric.⁴⁶

The process as used in this country and as adopted by the official agricultural chemists is conducted as follows:⁴⁷

390. Removal of the Alcohol.—The saponification is accomplished in the manner already described (382, 383), and when alcoholic potash is used proceed as follows:

The stopper having been laid loosely in the mouth of the flask, the alcohol is removed by dipping the flask into a steam-bath. The steam should cover the whole of the flask except the neck. After the alcohol is nearly removed, frothing may be noticed in the soap, and to avoid any loss from this cause or any creeping of the soap up the sides of the flask, it should be removed from the bath and shaken to and fro until the frothing disappears. The last traces of alcohol vapor may be removed from the flask by waving it briskly, mouth down, to and fro, or better by a current of carbon dioxid free air.

Dissolving the Soap.—After the removal of the alcohol the soap should be dissolved by adding 135 cubic centimeters of

⁴⁴ Reichert, *Zeitschrift für analytische Chemie*, 1879, **18** : 68.

⁴⁵ Meissl, *Dingler's Polytechnisches Journal*, 1879, **233** : 229.

⁴⁶ Lewkowitsch, *Chemical Technology and Analysis of Oils, Fats, and Waxes*, 4th Edition, 1909, **1** : 332.

⁴⁷ Division of Chemistry, *Bulletin* 46 (revised), 1899 : 46.

Bureau of Chemistry, *Bulletin* 107 (revised), 1912 : 139.

recently boiled distilled water, or 132 cubic centimeters when aqueous potassium hydroxid has been used for saponification, and warming on the steam-bath, with occasional shaking, until the solution of the soap is complete.

Setting Free the Fat Acids.—When the soap solution has cooled to about 60° or 70°, the fat acids are separated by adding five cubic centimeters of sulfuric acid made by diluting 200 cubic centimeters of the strongest acid to one liter or eight cubic centimeters when aqueous potassium hydroxid has been used for saponification.

Melting the Fat Acid Emulsion.—The flask is restoppered as in the first instance and the fat acid emulsion melted by replacing the flask on the steam-bath. According to the nature of the fat examined, the time required for the fusion of the fatty acid emulsions may vary from a few minutes to several hours.

The Distillation.—After the fat acids are completely melted, which can be determined by their forming a transparent, oily layer on the surface of the water, the flask is cooled to room temperature, and a few pieces of pumice stone added. The pumice stone is prepared by throwing it, at a white heat, into distilled water, and keeping it under water until used. The flask is connected with a glass condenser, Fig. 95, slowly heated with a naked flame until ebullition begins, and then the distillation continued by regulating the flame in such a way as to collect 110 cubic centimeters of the distillate in, as nearly as possible, 30 minutes. The distillate should be received in a flask accurately marked at 110 cubic centimeters.

Titration of the Volatile Acids.—The 110 cubic centimeters of distillate, after thorough mixing, are filtered through perfectly dry filter paper, 100 cubic centimeters of the filtered distillate poured into a beaker holding about a quarter of a liter, half a cubic centimeter of phenolphthalein solution added and deci-normal barium hydroxid solution run in until a red color is produced. The contents of the beaker are then returned to the measuring flask to remove any acid remaining therein, poured again into the beaker, and the titration continued until the red color produced remains apparently unchanged for two or three

minutes. The number of cubic centimeters of decinormal barium hydroxid solution required should be increased by one-tenth to represent the entire distillate.

The number thus obtained expresses, in cubic centimeters of

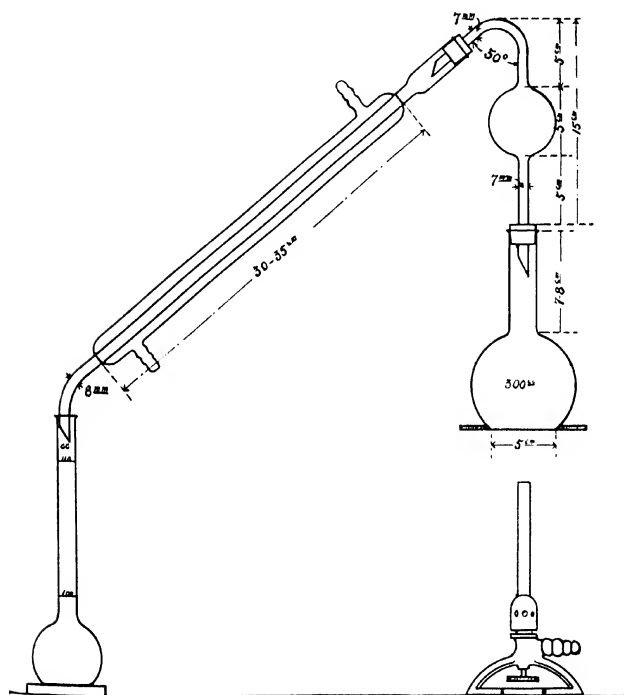


Fig. 95.—Apparatus for the Distillation of Volatile Acids.

decinormal alkali solution, the volatile acidity of the sample. If this be divided by the number of grams of fat originally taken and multiplied by five the Reichert-Meissl number is obtained. In each case blank distillations of the reagents used should be conducted under identical conditions, especially when alcoholic alkali is used for saponification. It is difficult to secure alcohol which

will not yield a trace of volatile acid in the conditions named. The quantity of decinormal alkali required to neutralize the blank distillate is to be deducted from that obtained with the sample of fat.

In as much as it is at present impossible to remove all the volatile without the decomposition of some of the non-volatile acids the various methods for the determination of the Reichert-Meissl number are empirical and must be closely adhered to in every detail if the results are to be comparable with figures found in the literature. The dimensions given in Fig. 95 are those adopted for the apparatus to be used in the Reichert-Wollny method, official with the Society of Public Analysts and the Government Laboratory of Canada.⁴⁸ This method is as follows:

Reichert-Wollny Method.—Five grams of fat are put into a flask of the form shown in Fig. 95, and two cubic centimeters of a caustic soda solution, made by dissolving 98 per cent. NaOH in an equal weight of recently boiled water; and 10 cubic centimeters of 92 per cent. alcohol added. The mixture is heated for 15 minutes under a reflux condenser on a water-bath and then the alcohol evaporated by heating the flask on the bath for half an hour or more. One hundred cubic centimeters of water (previously boiled for 10 minutes) are added and the soap heated until it is entirely dissolved. Forty cubic centimeters of normal sulfuric acid and a few pieces of pumice stone are then added and the flask at once connected to the distilling apparatus. The distillation is carried on as indicated under previous methods, and the titration and calculation, after subtracting the value of the blank, made as above.

Leffmann and Beam⁴⁹ have proposed the use of a glycerol soda instead of one made with alcohol to obviate the formation of ethyl esters during saponification which Wollny⁵⁰ stated might cause a loss of eight per cent. of volatile acids. The method as adopted by the Official Agricultural Chemists is as follows:⁵¹

⁴⁸ The Analyst 1900, **25** : 310.

⁴⁹ The Analyst 1891, **16** : 153.

⁵⁰ Journal of the Society of Chemical Industry 1887, 831.

⁵¹ Bureau of Chemistry, Bulletin 107, revised, 1912 : 139.

Add 20 cubic centimeters of the glycerol soda (made by adding 20 cubic centimeters of the strong caustic soda mentioned under Reagents (384) to 180 cubic centimeters of pure glycerol) to five grams of the fat in a flask, weighed as in the previous method, and heat over a naked flame or hot asbestos plate until complete saponification takes place, as is shown by the mixture becoming perfectly clear. If foaming occur, shake the flask gently.

Add 135 cubic centimeters of recently boiled water, drop by drop, at first, to prevent foaming, and five cubic centimeters of the dilute sulfuric acid (1-5), distil without previous melting of the fatty acids, and titrate the volatile acids as in the previous method, correcting results by the figure obtained in a blank experiment.

391. Determination of Soluble and Insoluble Fat Acids.—The volatile fat acids are more or less soluble in water, while those which are not distillable in a current of steam are quite insoluble. It is advisable, therefore, to separate these two classes of fat acids, and the results thus obtained are perhaps more decidedly quantitative than are given by the distillation process just described. The methods used for determining the percentage of insoluble acids are essentially those of Hehner.⁵² Many variations of the process have been proposed, especially in respect of the soluble acids.⁵³

The process approved by the Association of Official Agricultural Chemists is as follows:

Soluble Acids.—Place the flask used in the saponification determination (382) on a water-bath and evaporate the alcohol. Add such an amount of half-normal hydrochloric acid that its volume plus the amount used in titrating for the saponification number will be one cubic centimeter in excess of the amount required to neutralize the 50 cubic centimeters of alcoholic potash added.

⁵² *Zeitschrift für Analytische Chemie*, 1877, **16** : 145; 1879, **18** : 68.

Division of Chemistry, Bulletin 13, part 1, 1887 : 53; *The Analyst*: 1877, **1** : 147.

⁵³ *Journal of the Society of Chemical Industry*, 1888, **7** : 697; *American Chemical Journal*, 1888, **10** : 322; *Lewkowitsch, Oils, Fats, Waxes*, 4th Edition, 1909, **2** : 667.

Connect the flask with a condensing tube, three feet long and made of small glass tubing.

The flask is connected with a reflux condenser and placed on the steam-bath until the separated fat acids form a clear stratum on the upper surface of the liquid. Fill the flask to the neck with hot water, and cool in ice-water.

The fat acids having quite solidified, the liquid contents of the flask are poured through a dry weighed filter into a liter flask, taking care not to break the cake. Between 200 and 300 cubic centimeters of hot water are brought into the flask, the cork with the condenser reinserted and the flask placed on the steam-bath until the cake of acid is thoroughly melted. During the melting the flask should occasionally be agitated with a rotary motion in such a way that its contents are not made to touch the cork. When the fat acids have again separated into an oily layer, the flask and its contents are cooled in ice-water and the liquid filtered through the same filter into the same liter flask as before. This treatment with hot water, followed by cooling and filtration of the wash water, is repeated three times, the washings being added to the first filtrate. The mixed washings and filtrate are titrated with decinormal sodium hydroxid, using phenolphthalein as indicator. The number so obtained represents the volume of decinormal sodium hydroxid neutralized by the soluble fat acids of the fat, plus that corresponding to the excess of the standard acid used, *viz.*, one cubic centimeter. The number is therefore to be diminished by five, corresponding to the excess of one cubic centimeter of half-normal acid. This corrected volume multiplied by 0.0088 gives the weight of soluble acids as butyric acid in the amount of fat saponified; divide this by the amount of fat originally employed to obtain the percentage of soluble acids.

Insoluble Acids.—The flask containing the cake of insoluble fat acids from the above determination and the paper through which the soluble fat acids have been filtered are allowed to drain and dry for 12 hours, when the cake, together with as much of the fat acids as can be removed from the filter paper, is transferred to a weighed glass evaporating dish. The funnel, with the filter, is then placed in an Erlenmeyer and the paper thor-

oroughly washed with absolute alcohol. The flask is rinsed with the washings from the filter paper, then with pure alcohol and the rinsings and filtrate transferred to the evaporating dish. The dish is placed on the steam-bath until the alcohol is evaporated, dried for two hours at 100°, cooled in a desiccator and weighed. It is again placed in the air-bath for two hours, cooled as before and weighed. If there be any considerable decrease in weight, reheat two hours and weigh again. The final weighing gives the weight of insoluble fat acids in the sample, from which the percentage is easily calculated.

The quantity of non-volatile and insoluble acids in common glycerids is from 95 to 97 parts in 100. The glycerids yield almost the same proportion of fat acids and glycerol when the acids are insoluble and have high molecular weights. When the acids are soluble and the molecular weight low the proportion of acids decreases and that of glycerol increases.

In the following table, due to DeSchepper and Geitel, will be found the data secured by quantitative saponification and separation of soluble and insoluble acids found in the more common glycerids:

Glycerid	Fat acid	Molecular weight of		Yield per 100 parts of glycerid	
		Glycerid	Fat acid	Fat acid	Glycerol
Stearin	Stearic	890	284	95.73	10.34
Olein	Oleic	884	282	95.70	10.41
Palmitin	Palmitic	806	256	95.28	11.42
Myristin	Myristic	722	228	94.47	12.74
Laurin	Lauric	638	200	94.95	14.42
Caprin	Capric	594	172	93.14	15.48
Caproin	Caproic	386	116	90.16	23.83
Butyryn	Butyric	302	88	87.41	30.46

The general expression for the saponification of a neutral fat is $C_3H_5O_2.R_3 + 3H_2O = 3R.OH + C_3H_8O_3$, in which R represents the acid radicle. It is evident from this that the yield of more than 100 parts of fat acids and glycerol given by glycerids is due to the absorption of water during the reaction.

392. Formulas for General Calculations.—For calculating the theoretical yields of fat acids and glycerol, the following general formulas may be used:

Let M = the molecular weight of the fat acid:

K = saponification value:

F = the quantity of free fat acids in the glycerid:

N = the quantity of neutral fat in the glycerid:

A = the number of milligrams of potassium hydroxid required to saturate the free acid in one gram of the sample.

The free acid is determined by the method given below.

M grams of a fat acid require 56100 milligrams of potassium hydroxid for complete neutralization while F grams corresponding to 100 grams of fat are saturated by $100 \times A$ milligrams of the alkali.

Then $M : 56100 = F : 100 A$.

Whence $F = \frac{AM}{561} \dots \dots \dots (1)$.

Likewise since M grams of fat acid require the quantity of potassium hydroxid mentioned above we have:

$1 : K = M : 56100$,

Whence $M = \frac{56100}{K} \dots \dots \dots (2)$.

Substituting this value of M in (1) we have

$F = \frac{A \times 56100}{561 \times K} = \frac{100 A}{K} \dots \dots \dots (3)$.

It is evident that it is not necessary to calculate the acid value (A) of the sample and the saponification value (K) of the free fat acids, the ratio $\frac{A}{K}$ alone being required. It will be sufficient therefore to substitute for A and K the number of cubic centimeters of alkali solutions required for one gram of the fat and one gram of the fat acids, respectively. If a and b represent these numbers the formula may be written

$F = \frac{100 a}{b} \dots \dots \dots (4)$;

and $N = 100 - F = 100 - \frac{100 a}{b} \dots \dots \dots (5)$.

To simplify the determinations, it may be assumed that the free fat acids have the same molecular weight as those still in combination with the glycerol in any given sample. On this assumption, the process may be carried on by determining the acid value A and the saponification value K for the total fat acids. The mean molecular weight M , the percentage of free fat acids F , and the proportion of neutral fat N , may then be calculated from the formulas (2), (3), (4), and (5).

Further, let G = the quantity of glycerol and L that of fat acids obtainable from one gram of neutral fat, that is, $\frac{1}{100}$ of H the percentage of total fat acids.

The molecular weight of the neutral fat in each case is $3M + 38$. Therefore, $3M + 38$ parts of neutral fat yield $3M$ parts of fat acids and 92 parts of glycerol ($C_3H_8O_3 = 92$).

$$\text{Then } L = \frac{H}{100} = \frac{3M}{3M + 38} \dots\dots\dots (6);$$

$$\text{and } G = \frac{92}{3M + 38} \dots\dots\dots (7).$$

N per cent. of neutral fat yields, therefore, on saponification, the following theoretical quantities of fat acids F , and glycerol G expressed as parts per hundred.

$$F = N \times \frac{3M}{3M + 38} \dots\dots\dots (8);$$

$$\text{and } G = N \times \frac{92}{3M + 38} \dots\dots\dots (9).$$

Formula (9) expresses also the total yield of glycerol from any given sample. For a further discussion of this part of the subject a work of a more technical character may be consulted.⁵⁴

393. Determination of a Free Fat Acid in a Fat.—The principle of the method rests upon the comparative accuracy with which a free fat acid can be titrated with a set alkali solution when phenolphthalein is used as an indicator. Among the many methods of manipulation which the analyst has at his command there is probably none more simple and accurate than that depending

⁵⁴ Lamborn, *Modern Soaps, Candles and Glycerin*, 1906: 14.

on the solution of the sample in alcohol, ether, chloroform, or carbon tetrachlorid. As the solvent itself may be slightly acid it should first be neutralized with weak caustic soda solution. Either an aqueous or alcoholic solution of the alkali may be used, preferably the latter. The alkaline solution may be approximately or exactly decinormal, but it is easier to make it approximately so and to determine its real value before each operation by titration against a standard decinormal solution of acid. About 20 grams of the sample and 50 cubic centimeters of the solvent will be found convenient quantities. The official method is conducted as follows:⁵⁵

Free Fatty Acids—Provisional.—Place 20 grams of fat or oil in a flask, add 50 cubic centimeters of 95 per cent. alcohol which has been neutralized with weak caustic soda, using phenolphthalein as indicator, and heat to the boiling point. Agitate the flask thoroughly in order to dissolve the free fatty acids as completely as possible. Titrate with tenth-normal alkali, agitating thoroughly until the pink color persists after vigorous shaking.

Express results either as percentage of oleic acid, as acid degree (cubic centimeters of normal alkali required to neutralize the free acids in 100 grams of oil or fat), or as acid value (milligrams of potassium hydroxid required to saturate the free acids in one gram of fat or oil).

One cubic centimeter of tenth-normal alkali = 0.0282 gram of oleic acid.

Example.—Ten grams of rancid olive oil dissolved in alcohol-ether require three and eight-tenths cubic centimeters of a solution of alcoholic potash to saturate the free acid present. When titrated with decinormal acid the potash solution is found to contain 25.7 milligrams of potassium hydroxid in each cubic centimeter. The specific gravity of the oil is 0.917 and the weight used therefore 9.17 grams. Then the total quantity of potassium hydroxid required for the neutralization of the acid is $25.7 \times 3.8 = 97.7$ milligrams.

⁵⁵ Division of Chemistry Bulletin 107, revised, 1910 : 142.

Allen, Commercial Organic Analysis, 3rd Edition, 2 : 105.

The acid value A is therefore:

$$A = \frac{3.8 \times 25.7}{9.17} = 10.6$$

It is customary to regard free acid as oleic, molecular weight 282. On this assumption the percentage of free acids in the above case is found by the formula

$$A \text{ (per cent.)} = \frac{3.8 \times 25.7 \times 282}{561 \times 9.17} = 5.35$$

394. Coloration Produced by Reagents.—When oils and fats are mixed with oxidizing reagents, such as sulfuric and nitric acids, the glycerids are partly decomposed with the production of colors which have some analytical significance. The most simple method of applying these tests is by the use of a thick porcelain plate provided with small cup-shaped depressions for holding the few drops of material required. Two or three drops of the oil under examination are placed in each of the cups, a like quantity of the oxidizing reagent added, and the mixture stirred with a small glass rod. The colors produced are carefully noted and the mixture is allowed to remain at room temperature for at least 12 hours in order that the final tint may be observed. The sulfuric acid used for this reaction should have a specific gravity of one and seven-tenths and the nitric acid should have the usual commercial strength of the strongest acid. Pure lard, when treated with sulfuric acid, as above described, shows but little change of color while the vegetable oils mostly turn brown or black. In addition to the reagents mentioned many others, including sulfuric and nitric acids, sulfuric acid and potassium bichromate, chlorin, ammonia, hydrogen peroxid, sodium hydroxid, calcium polysulfid, zinc chlorid, stannic chlorid, phosphoric acid, mercuric nitrate and aqua regia are used. Only a few of these tests seem to have sufficient analytical importance to merit any detailed description.⁵⁶

395. Coloration in Large Masses.—Instead of applying the color test in the small way just described, larger quantities of the fat may be used, either in the natural state or after solution in petro-

⁵⁶ Division of Chemistry, Bulletin 13, part 4, 1899: 415, 448.

leum or other solvent. For this purpose about 10 cubic centimeters of the oil are shaken with a few drops of sulfuric acid or sulfuric and nitric acids. Lard, when thus treated (five drops of sulfuric acid to 10 cubic centimeters of lard) shows practically no coloration. When treated with an equal volume of sulfuric acid and shaken, the lard on separating has a brown-red tint.⁵⁷

⁵⁷ Taylor, Annual Report U. S. Department of Agriculture, 1877 : 622.

PART FIFTH

SEPARATION AND ESTIMATION OF BODIES CONTAINING NITROGEN.

396. Nature of Nitrogenous Bodies.—The nitrogenous bodies, valuable as foods, belong to the general class of proteins and albuminoids. They are composed chiefly of carbon, hydrogen, oxygen, sulfur and nitrogen. Some of them, as lecithin and nuclein, contain phosphorous instead of sulfur, but these resemble the fats rather than the proteins.

Nitrogenous organic bodies of the class mentioned above are designated by the general name proteins. The term albumin is restricted in a physiological sense to a certain class of proteins. The term albuminoid is often used synonymously, as above, for proteins, but, more strictly speaking, it should be reserved for that class of bodies such as gelatin, mucin, keratin and the like, not really proteins, but, nevertheless, closely resembling them. These form the principal organic constituents of skeleton structure of animals and of the skin, hair, nails, hoofs and horns. In chemical composition the proteins are characterized by the relative constancy of their nitrogen content, the mean percentage of this element being about 16, but greatly varying in some instances from that number.

397. Classification of Proteins.—Many classifications of the proteins have been given based on physical, chemical and physiological characteristics. In respect of origin, they are divided into two great classes, *viz.*, vegetable and animal. In respect of their physical and chemical properties the following classification of the proteins may be made:⁵⁸

I. *Simple Proteins.*—These are bodies which yield on hydrolysis only α -amino acids or their derivatives.

⁵⁸ Proceedings of the American Physiological Society, 1907-8, Twentieth Annual Meeting : 27.

(a) *Albumins*.—These are proteins soluble in water and not precipitated from their aqueous solutions by sodium chlorid or magnesium sulfate. They are easily coagulated by heat and are represented by three great classes, *viz.*, egg-, serum- and lactalbumin.

Egg albumin occurs in the white of egg; serum albumin is found in the serum of the blood. Vegetable albumins have been prepared from wheat, rye, potatoes, and papaws (*Carica Papaya*). These vegetable albumins are coagulated by heat at about 70° and are not precipitated by the salt solutions named above, nor by acetic acid. The myrosin of mustard seeds also resembles vegetable albumin.

(b) *Globulins*.—These bodies are insoluble in water, soluble in dilute solutions of neutral salts, but precipitated therefrom by saturation with sodium chlorid or magnesium sulfate. They are coagulated by heat. Among others belonging to this group are serum globulin, fibrinogen, myosin, crystallin, and globin.

Serum globulin is found in the serum of blood; cell globulin is found in lymph cells; fibrinogen occurs in the blood plasma; plasmin, in blood plasma; myosin, in dead muscles; vitellin, in the yolk of eggs; crystallin, in the lens of the eye; haemoglobin, in the red pigment of the blood; haemocyanin, in the blood of certain low grade animals.

Vegetable globulins are found in the cereals, leguminous plants, papaws and other vegetables, and are divided into two groups, myosins and paraglobulins. The vegetable myosins coagulate at from 55° to 60° and are precipitated from a saline solution by removing the salt by dialysis. In this form, however, they lose their true nature as globulins, becoming insoluble in weak saline solutions.

The vegetable paraglobulins are coagulated at from 70° to 75°. Vegetable vitellin, which is not included in this classification, can be obtained in a crystalline form and of remarkable purity.⁵⁹

(c) *Glutelins*.—Simple proteins insoluble in all neutral solvents but readily soluble in very dilute acids and alkalies. These substances occur abundantly in the seeds of cereals.

⁵⁹ Barbieri: *Journal für praktische Chemie*, neue Folge, 1878, 18 114.

(d) *Alcohol Soluble Proteins*.—Simple proteins soluble in 70-80 per cent. alcohol, but insoluble in water, absolute alcohol and other neutral solvents.

(e) *Histones*.—Soluble in water, insoluble in very dilute ammonia and, in the absence of ammonium salts, insoluble even in an excess of ammonia. They give a coagulum on heating, soluble in dilute acids.

(f) *Ptomaines*.—Polypeptides soluble in water, not coagulated by heat, are strongly basic forming stable salts with mineral acids.

(g) *Albuminates*.—This name was formerly given to the compounds of the proteins with metallic oxids or bases, and also to acid and alkali albumins. They are insoluble in water or dilute neutral salts, but easily soluble in strong acids or alkalies. Casein is a type of this group.

Acid albumin is made from egg albumin by treatment with hydrochloric acid; alkali albumin is formed in egg albumin by the action of a dilute alkali; trinitroalbumin is formed from dry albumin by treatment with nitric acid; casein or caseinogen, is the chief protein in milk.

The chief vegetable albuminates are legumin and conglutin. Legumin is a vegetable casein and occurs chiefly in peas, beans and other leguminous seeds. It is prepared by extracting the meal of the seeds mentioned with dilute alkali, filtering the extract, precipitating with acetic acid, washing the precipitate with alcohol, and drying over sulfuric acid. Treated with sulfuric acid it yields leucin, tyrosin and glutamic and aspartic acids. Conglutin is prepared in a similar manner from almonds.

It is probable that these bodies do not exist as such in the fresh seeds in question but are produced therein from the other proteins by the alkali used in extraction. A further description of vegetable proteins will be found in the special paragraphs devoted to the study of these bodies in the principal cereals.

II. *Conjugated Proteins*.—These are substances which contain the protein molecule united to some other molecule not a salt.

(a) *Nucleoproteins*.—These are compounds of one or more protein molecules with nucleic acid.

(b) *Glycoproteins*.—These are compounds of protein molecules with carbohydrates or carbohydrate containing bodies.

(c) *Phosphoproteins*.—These are possibly esters of some phosphoric acid and protein, but excluding lecithin.

(d) *Haemoglobins*.—These are compounds of proteins with haematin or some similar substance.

(e) *Lecithoproteins*.—These are compounds of the protein molecule with lecithin forming lecithous or phosphotides.

III. *Derived Proteins*.—Primary protein derivatives are apparently formed through hydrolytic changes which involve only slight alterations of the protein molecule. They are divided into three classes, viz.:

(a) *Proteins*.—Insoluble products which apparently result from the incipient action of water, very dilute acids or enzymes.

(b) *Metaproteins*.—These are more advanced alteration products such as the incipient albuminates mentioned above and also like acid products, being soluble in weak acids or alkalies but insoluble in neutral solvents.

(c) *Coagulated Proteins*.—Insoluble products resulting from the action of heat and strong alcohol on soluble proteins.

IV. *Secondary Protein Derivatives*.—These are products derived from the more complete hydrolysis of the protein molecules. For convenience they are divided into the following classes:

(a) *Proteoses*.—This name is applied to proteins which are not coagulated by heat, but most of them are precipitated by saturated solutions of neutral salts. They are also precipitated by nitric acid. They are formed from other proteins by the action of proteolytic ferments. The albumoses represent this group.

Protoalbumose is soluble in distilled water and weak saline solutions and is precipitated by mercuric chlorid and copper sulfate.

Heteroproteose is insoluble in distilled water, but soluble in weak saline solutions, from which it separates when the salts are removed by dialysis. Deuteroalbumose is soluble in distilled water and saline solutions and is not precipitated on saturation

with sodium chlorid. It is thrown out by mercuric chlorid *but not by copper sulfate*.

Vegetable proteoses are known as phytalbumoses, two of which have been found in the juice of the papaw mentioned above. They have also been found in cereals.

(b) *Peptones*.—These bodies are very soluble in water but are not thrown out by heat, by saturation with neutral salts, nor by nitric acid. They are completely precipitated by tannin and by strong alcohol.

The peptones are the only soluble proteins which are not precipitated by saturation with ammonium sulfate. The principal animal varieties are hemi- and anti-peptones. These forms of proteins do not appear to exist as such in vegetable products but are produced in large quantities by treating other proteins with pepsin or pancreatin. In sprouting plants, there appears to be a widely diffused ferment capable of converting the proteins of the cotyledons into peptonoid bodies and thus fitting them for entering the tissues of the new plant.

Insoluble Proteins.—This class includes a miscellaneous collection of nitrogenous bodies not belonging to any of the definite groups already mentioned. Fibrin and gluten are types of these insoluble bodies. Fibrin is formed from the fibrinogen of fresh blood and causes coagulation. When washed free of red blood corpuscles it is a white elastic solid. It is insoluble in water and is converted into albumoses and peptones by trypsin and pepsin. It swells up when treated with a very weak one-tenth per cent. solution of hydrochloric acid and dissolves to acid albumin when heated therewith.

Gluten is the most important of the insoluble vegetable proteins and forms the chief part of the nitrogenous constituents of wheat. It is readily prepared by washing wheat flour in cold water, as will be described further on. It is probably a composite body formed by the process of extraction from at least two protein bodies, glutenin and gliatin, existing in wheat. When dried it forms a horny elastic mass of a yellow-gray color. Gluten is composed of two bodies, one soluble the other insoluble in alcohol.

398. Principal Albuminoids.—In this paragraph the term albuminoids is employed to include the bodies under (e) of the simple proteins described above. Some of them are of such importance as to merit description under their older classifications.*

Collagen.—The nitrogenous portions of connective tissues are largely composed of collagen. By boiling water it is converted into gelatin. It may be prepared from tendons as follows: The tendinous tissues are shredded as finely as possible and extracted with cold water to remove the soluble proteins. Thereafter they are subjected for several days to the action of lime water, which dissolves the cement holding the fibers together. The residual insoluble matter is washed with water, weak acetic acid, and again with water. The residue is chiefly collagen, mixed, however, with some elastin and nuclein. With dilute acids and alkalis collagen swells up after the manner of fibrin. The organic nitrogenous matter of bone consists largely of collagen, which is sometimes called ossein.

Gelatin.—When the white fibers of collagen, obtained as above, are subjected to the action of boiling water or of steam under pressure they dissolve and form gelatin. Isinglass is a gelatin made from the swimming bladder of the sturgeon or other fish. Glue is an impure gelatin obtained from hides and bones. Pure gelatin may be prepared from the commercial article by removing all soluble salts therefrom by treatment with cold water, dissolving in hot water and filtering into 90 per cent. alcohol. The gelatin separates in the form of white filaments and these are removed and dried. Gelatin is insoluble in cold but soluble in hot water. It is insoluble in alcohol, ether and chloroform. Its hot aqueous solutions deflect the plane of polarized light to the left. Its gyrodynic varies with temperature and degree of dilution and is also influenced by acids and alkalies. At 30° it is $[\alpha]_{D^{30}} = -130$.

Gelatin is not precipitated by acetic acid nor lead acetate solution, in which respect it differs from chondrin.

If boiled for a day, or in a short time if heated to 140° in a sealed tube, gelatin loses its power of setting and is split up into

* This book, First Edition, Vol. III : 413.

two peptonoid bodies, semi-glutin and hemi-collin. Gelatin is easily digested but cannot take the place of other proteids in nutrition.

Mucin.—This albuminoid, together with globulin, forms the principal part of connective tissue. It is also present in large quantities in mucus and is the chief lubricant of mucous membranes. It is extremely difficult to prepare mucin in a state of purity, and it is not certain that it has ever been accomplished. It is precipitated but not rendered subsequently insoluble by sodium chlorid, magnesium sulfate and alcohol. When boiled with sulfuric acid it yields leucin and tyrosin and, with caustic soda, pyrocatechin.

Mel- and Paralbumin.—Metalbumin is a form of mucin and differs from paralbumin by giving no precipitate when boiled. Both bodies yield reducing sugars when boiled with dilute sulfuric acid.

Nuclein.—The nitrogenous matters which form the nuclei of the ultimate cells are called nuclein. Nuclein resembles mucin in many physical properties but contains phosphorous. It is also, like mucin, resistant to pepsin digestion. The nuclein of eggs and milk probably contains iron. Nuclein is found also in cells of vegetable origin and in yeast and mildew.

Nucleoproteins.—These are bodies which yield both nuclein and albumin when boiled with water or treated with dilute acids or alkalis. Many nucleoproteins have the physical properties of mucus and the sliminess of the bile and of the synovial liquid is due to them. They are the chief nitrogenous constituent of all protoplasm.

Chondrin.—Chondrin is obtained from cartilage by boiling with water. The solutions of chondrin set on cooling in the manner of gelatin. They are precipitated by the same reagents used for throwing out gelatin and mucin. Chondrin is also levorotatory. By some authorities chondrin is regarded as a mixture of gelatin and mucin.

Elastin.—The elastic fibers of connective tissue are composed of this material. It can be prepared from the neck muscles by boiling with ether and alcohol to remove fats and then for a day

and a half with water to extract the collagens. The residue is boiled with strong acetic acid and thereafter with strong soda until the fibers begin to smell. It is then treated with weak acetic acid and for a day with dilute hydrochloric acid. The acid is removed by washing with water and the residue is elastin. There is no solvent which acts on elastin without decomposing it. It is digested by both pepsin and trypsin with the formation of peptones.

Kreatin.—This nitrogenous substance is found chiefly in hairs, nails, and horns. It is essentially an alteration protein product due to peripheral exposure. It is prepared by digesting the fine ground material successively with ether, alcohol, water and dilute acids. The residue is keratin. An imperfect aqueous solution may be secured by heating for a long time under pressure to 200°. It is also dissolved by boiling the materials mentioned above with alkalies, and when the solution thus obtained is treated with water, hydrogen sulfid is evolved, showing that the sulfur of the molecule is loosely combined.

Horn swells up when treated with dilute acetic acid and dissolves in the boiling glacial acid. When treated with hot dilute sulfuric acid it yields aspartic and volatile fat acids, leucin and tyrosin. Kreatin, when burning, gives off a characteristic odor as is perceived in burning hair.

Other Albuminoids.—Among the albuminoids of less importance may be mentioned neurokeratin, found in the medullary sheath of nerve fibers; chitin, occurring in the tissues of certain invertebrates; conchiolin, found in the shells of mussels and snails; spongin, occurring in sponges; fibroin, forming silk and spiders' webs; and hyalin or hyalogen, found in edible birds' nests.

The nitrogenous bases in flesh which are soluble in cold water, *viz.*, kreatin, kreatinin, carnin, sarkin and xanthin are not classed among the albuminoid bodies, since they have a much higher percentage of nitrogen than is found in true protein bodies, and are further differentiated from them by the absence of sulfur.

399. Other Forms of Nitrogen.—In addition to the proteins and albuminoids mentioned above, agricultural products may contain

nitrogen in the form of ammonia, amid nitrogen and nitric acid. The quantities of nitrogen thus combined are not large but often of sufficient magnitude to demand special study. In general, these bodies belong to transition products, representing stages in the transfer of nitrogen from the simple to complex forms of combination, or the reverse.

For instance, the nitrogen which finally appears in the proteins of a plant has entered its organism chiefly as nitric acid, and the nitric acid which is found in a vegetable product is therefore a representative of the quantity of unabsorbed nitrogen present in the tissues at the moment when the vital activity of the plant is arrested. In some instances, it is found that the absorption of nitrates by vegetable tissues takes place in far larger quantities than is necessary for their nutrition, and in these cases the excess of nitrates accumulates, sometimes to a remarkable extent. In a case cited in the reports of the Kansas Agricultural Experiment Station, where Indian corn was grown on ground which had been used for a hog pen, the quantity of potassium nitrate found in the dried stalks was somewhat remarkable. When one of the stalks was cut in two and tapped lightly upon a table, crystals of potassium nitrate were easily obtained in the form of fine powder. On splitting the cornstalk the crystals in the pith could be seen without the aid of a microscope. On igniting a piece of the dried stalk it burned rapidly with deflagration. The percentage of potassium nitrate in the dried material was 18.8. Cattle eating this fodder were poisoned.⁶⁰

In preserved meat products large quantities of oxidized nitrogen are often found, and these come from the use of potassium nitrate as a preserving and coloring agent. Ammonia is rarely found in vegetable tissues in greater quantities than mere traces, but may often exist in weighable amounts in animal products, especially those in a state of incipient decomposition.

Amid nitrogen is found rather constantly associated with protein matters in vegetable products. Asparagin and glutamin are instances of amid bodies of frequent occurrence. Betain and cholin are found in cottonseed.

⁶⁰ Kansas Experiment Station, Bulletin 49, 1895.

The occurrence of nitrogen, in the form of alkaloids, is of interest to agricultural chemists in this country, chiefly from its presence as nicotin in tobacco and from a toxicological point of view, but in other localities the production of alkaloids, as for instance in opium, tea and coffee, is a staple agricultural industry. The methods of separating and determining these forms of nitrogen will be given further on. This description can evidently not include an extended compilation of the methods of separating and determining alkaloidal bodies, with the exception of those with which the agricultural analyst will be called upon frequently to deal, *viz.*, nicotin and caffen and nitrogenous bases such as betain and cholin.

QUALITATIVE TESTS FOR NITROGENOUS BODIES.

400. Nitric Acid.—Any nitric acid or nitrate which an agricultural product may contain may be leached out by treating the fine-ground material with cold water. From vegetable matters this extract is evaporated to a small bulk, filtered, if necessary, and tested for nitric acid by the usual treatment with ferrous sulfate and sulfuric acid. In the case of vegetable substances there will not usually be enough of organic matter to interfere with the delicacy of the reaction, but in animal extracts this may occur. Colored extracts should be decolorized with animal char (boneblack) before they are subjected to examination. It is not well to attempt to remove the organic matters, but, since they are more insoluble in water than the nitrates, the solution containing both may be evaporated to dryness and treated with a quantity of cold water insufficient for complete solution. The nitrates will be found in the solution obtained in a larger proportionate quantity than before.

401. Amid Nitrogen.—One or more atoms of the hydrogen in ammonia may be replaced by acid or basic bodies (alcohol radicals). In the former cases amids, in the latter amins result. In the ratio of displacement there are formed primary, secondary, and tertiary bodies determined by the number of hydrogen atoms replaced. The primary amids are the only ones of these bodies that are of interest in this connection.

The amids are easily decomposed, even on heating with water and the more readily with acids and alkalies, the amido radicle being converted into ammonia. A type of these reactions is given below.



On boiling an amid with hydrochloric acid, the ammonia is procured as chlorid whence it is easily expelled by heating with an alkali. In a body free of ammonia, an amid is easily detected by subjecting the substance containing it to the action of hot hydrochloric acid, filtering, neutralizing the free acid with sodium hydroxid, adding an excess thereof and distilling into an acid.⁶¹ In case the quantity of ammonia produced is very small it may be detected by the Nessler reagent.⁶² Amids are soluble in a fresh, well washed preparation of cupric hydrate suspended in water. The hydrate also passes into solution forming a liquid of a deep blue color.

If amids be added to a cold solution of potassium nitrate in sulfuric acid free nitrogen is evolved.

402. Ammoniacal Nitrogen.—This combination of nitrogen may be detected by distilling the sample, or an aqueous extract thereof, with magnesia or barium carbonate. The ammonia is collected in an acid and detected therein by the usual qualitative reactions.

403. Protein Nitrogen.—There are a few general qualitative reactions for protein nitrogen and some special ones for distinct forms thereof. Below will be given a few of those reactions which are of most importance to the agricultural analyst:

Conversion into Ammonia.—All protein matters are converted into ammonia on boiling with strong sulfuric acid in presence of an oxygen carrier. Mercury is the substance usually selected to effect the transfer of the oxygen. Bodies which are found to be free of nitrates, ammonia and amids, are subjected directly to oxidation with sulfuric acid, and the ammonia produced thereby is distilled and detected in the manner already suggested. If nitrogen be present in the form of ammonia, amids and nitrates,

⁶¹ Principles and Practice of Agricultural Analysis, 1908, 2 [2] : 387.

⁶² Principles and Practice of Agricultural Analysis, 1908, 2 [2] : 481.

the substance may be heated with an acid, hydrochloric or acetic, thrown on a filter, washed with hot dilute acid and the residue tested as above for protein nitrogen.

Biuret Reaction.—When protein matter is dissolved in sulfuric acid, the solution, made alkaline with potassium hydroxid and treated with a few drops of a solution of copper sulfate, gives a violet coloration. This is commonly known as the biuret reaction, because the substance $C_2H_6N_3O_2$, biuret, left on heating urea to 160° gives the coloration noted in the conditions mentioned.

It has been found by Bigelow, that if a solution is to be examined containing a very small amount of a protein or similar body, the copper sulfate solution should not contain more than four grams of $CuSO_4 \cdot 5H_2O$ in 100 cubic centimeters of water, and the test should first be made by adding to the solution one or two drops of this copper sulfate solution, and then a strong excess of potassium or sodium hydroxid. The test may be repeated, using from one-half to two cubic centimeters of the copper sulfate solution, according to the amount of protein present. If too much of the copper sulfate solution be employed its color may conceal that of the reaction.

Heating to the boiling point sometimes makes the violet color more distinct.

If a solid is to be examined it is first suspended in water, and in this state treated in the same manner as a solution. If the solution is not complete, the mixture should be filtered when the color produced may be observed in the filtrate.

Proteoses and peptones give a red to red-violet and other proteins a violet to violet-blue coloration.

Xanthoproteic Reaction.—Strong nitric acid produces a yellow coloration of protein matter, which is intensified on warming. On treating the yellow mixture with ammonia in slight excess the color is changed to an orange or red tint.

404. Qualitative Tests for Albumin.—Albumin is one of the chief proteins and exists in both animal and vegetable substances. It is soluble in cold water and may therefore be separated from many of its nearly related bodies which are insoluble in that menstruum. In aqueous solutions its presence may be

determined by the general reactions for protein matters given above or by the following tests:

Precipitation by Heat.—Albumin is coagulated by heat. Vegetable albumins become solid at about 65° and those of animal origin at a somewhat higher temperature (75°). Some forms of animal albumin, however, as for instance that contained in the serum, coagulate at a lower temperature.

Precipitation by Acids.—Dilute acids also precipitate albumins especially with the aid of heat. Practically all the albumins are thrown out of solution by application of heat in the presence of dilute acids.

Mercuric Salts.—Acid mercuric nitrate containing a trace of nitrous acid and a mixture of mercuric chlorid, potassium iodid and acetic acid completely precipitate all albuminous matters.⁶³

Heating albumin with the mercuric nitrate reagent producing a yellow or red color is known as Millon's reaction.

405. Qualitative Test for Peptones and Albuminates.—When peptones and albuminates are dissolved in an excess of glacial acetic acid and the solution treated with sulfuric acid a violet color is produced and also a faint fluorescence.

Separation of Peptones and Albumoses.—In a solution of peptones and albumoses the latter may be precipitated by saturating the solution with finely powdered zinc or ammonium sulfate.

Action of Phosphotungstic Acid.—All protein matters in aqueous, alkaline or acid solutions, are precipitated by sodium phosphotungstate in a strongly acid solution. Acetic, phosphoric, or sulfuric acid may be used for producing the required acidity, preference being given to the latter.

Action of Trichloroacetic Acid.—In the precipitation of albumin by trichloroacetic acid, there is formed a compound of the two bodies which to 100 parts of albumin has 26.8 parts of the trichloroacetic acid.

The different albuminoid bodies obtained by preprecipitation behave in a similar manner. There are formed flocculent precipitates insoluble both in dilute and concentrated acids in the

⁶³ Wiley, American Chemical Journal, 1884-85, 6 : 293.

cold and also at a high temperature, with the exception of the hemialbumose compound.⁶⁴

Albumin peptone gives with the acid named in concentrated solution a precipitate easily soluble in an excess of the reagent. In the analysis of cows' milk but not of human milk, this acid can be used for the estimation of the albuminoid substances. With both kinds of milk it can be used for the estimation of the albumin after the removal of the casein.

By means of trichloroacetic acid it is possible to separate albumin peptone from mucus and mucus peptone. A similar reaction is also produced by dichloroacetic acid, but the reaction with this last reagent is less delicate than with the other. Neither mucus nor albumin is precipitated by chloroacetic acid.

406. Action of Albumins on Polarized Light.—Many of the albumins and albuminates, when in solution, strongly deflect the plane of polarized light to the left.⁶⁵

The gyrodynes of some of the albumins and albuminates are given below :

Serum Albumin (man)	(α) _D = — 62° to — 64.59°
“ “ (dog)	(α) _D = — 43.77°
“ “ (horse)	(α) _D = — 47.4° & — 60.05°
Egg albumin	(α) _D = — 30.70° to — 29.4°
Serum globulin	(α) _D = — 47.8°
Milk albumin	(α) _D = — 36.4° to — 38.98° ⁶⁶

The figures (α)_D vary greatly with different investigators and with the sources of the albumins.

Our knowledge of the rotatory numbers of the proteins and allied bodies is too fragmentary to be of any great help in analytical work. In practice, the rotatory power of these bodies becomes a disturbing force in the determination of milk sugar.⁶⁷

⁶⁴ Obermayer : *Chemiker-Zeitung, Repertorium*, 1889, **13** : 269.

⁶⁵ Hoppe-Seyler, *Handbuch der physiologisch- und pathologisch-chemischen Analyse*, 1909 [8] 397, 400, 401.

⁶⁶ Abderhalden, *Biochemisches Handlexikon*, (1910) **4**, Part 1 : p. 60, 66, 79 and 82.

⁶⁷ Wiley, *American Chemical Journal*, 1884-85, **6** : 289.

A further study of this property of certain proteins may lead to analytical processes for their detection and determination, but no reliable methods for this can now be recorded.

407. Alkaloidal Nitrogen.—Only a general statement can be made here in respect of the detection of alkaloidal nitrogen in vegetable or animal tissues. Alkaloids are not found in healthy animal tissues and the description of methods for isolating and detecting ptomaines is foreign to the purpose of this work. In vegetable tissues the presence of alkaloids may be established by the following methods of examination.

The fine-ground tissues are made to pass a sieve of half millimeter mesh and when suspended in water are acidified with sulfuric. The mixture is then thoroughly extracted by shaking in a separatory funnel with petroleum ether, benzene and chloroform, successively. Some resins, glucosids and a few alkaloidal bodies not important here are extracted by this treatment.

The residue is made distinctly alkaline with ammonia and treated as above with the same solvents. In the solution obtained as last mentioned nearly all the alkaloidal bodies found in plants are contained.

All the alkaloids in a plant may be obtained by digesting the finely divided material with dilute sulfuric acid. The acid solution thus obtained is made nearly neutral with ammonia or magnesia, concentrated to a sirup, and gums, mucilage, etc., thrown out by adding about three volumes of 95 per cent. alcohol. The alkaloids are found in the filtrate. The alcohol is evaporated from the filtrate and the residue tested for alkaloids by group reagents.⁶⁸ Potassium mercuric iodid and phosphotungstic and molybdic acids are types of these reagents.

The same group reagents may also be applied to the extracts obtained with petroleum ether, benzene and chloroform, in all cases, after the removal of the solvents by evaporation.

⁶⁸ Dragendorff. *Plant Analysis*, 1884, 55.

ESTIMATION OF NITROGENOUS BODIES IN AGRICULTURAL PRODUCTS.

408. Total Nitrogen.—Any one of the methods heretofore described for the estimation of total nitrogen in soils or fertilizers is applicable for the same purpose to agricultural products. One among these, however, is so superior in the matter of convenience and certainty, as to make it preferable to any other. The moist combustion of the sample with sulfuric acid with subsequent distillation of the ammonia produced is the process which is to be recommended.⁶⁹

The usual precautions for securing a representative sample should be observed, but no further directions are needed. In all cases hereafter, where the estimation of nitrogen is enjoined, it is understood that the moist combustion process is to be used unless otherwise stated.

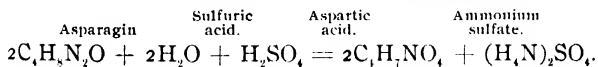
409. Estimation of Ammoniacal Nitrogen.—If the distillation of ammonia be accomplished with the aid of magnesia alba or barium carbonate it may be safely conducted on the finely ground materials or, in case of animal bodies, in as fine a state of subdivision as may be conveniently secured. Since the salts of ammonia are easily soluble in water they may be all obtained in aqueous solution, and the distillation of this solution with magnesia gives correct results. Experience has shown that the stronger alkalis, such as sodium and potassium hydroxids, cannot be safely used in the distillation of ammonia from mixtures containing organic nitrogenous materials because of the tendency of these bodies to decomposition, in the circumstances, yielding a portion of their nitrogen as ammonia. Barium carbonate acts with less vigor on non-ammoniacal nitrogenous matters than magnesia, and in some cases, as pointed out further on, may be substituted therefor with advantage. There is no danger of failing to obtain a part of the ammonia on distillation with magnesia provided the latter does not contain more than a trace of carbonate.⁷⁰

⁶⁹ Principles and Practice of Agricultural Analysis, 1908, 2, [2]: 346 et seq. Bureau of Chemistry, Bulletin 107, (revised) 1912: 5-11.

⁷⁰ Chemiker-Zeitung, 1896, 20: 151.

When no easily decomposable organic nitrogenous matters are present, the distillation may be conducted with the stronger alkalis in the manner prescribed.⁷¹ All the necessary details of conducting the distillation are found in the preceding volumes of this work.

410. Estimation of Amid Nitrogen.—In bodies containing no ammonia, or from which the ammonia has been removed by the method described in the preceding paragraph, the nitrogen in the amid bodies is converted into ammonia by boiling for about an hour with five per cent. sulfuric or hydrochloric acid. The ammonia thus produced is estimated in the usual manner after distillation over magnesia free of carbonate. The free acid is exactly neutralized with sodium or potassium carbonate before the addition of the magnesia. The results are given in terms of asparagin. The reaction which takes place in the decomposition of the amid body is indicated by the following equation:



Half of the nitrogen contained in the amid body is thus obtained as ammonia.

It is advisable to calculate all the amid nitrogen in agricultural products as asparagin.

411. Sachsse's Method.—A method for the determination of amid bodies by liberation of free nitrogen has been described by Sachsse and Kormann.⁷² It is based on the reaction which takes place when amid bodies are brought into contact with nitrates in presence of an acid. The mixture of the reagents by which the gas is set free is accomplished in the apparatus shown in Fig. 96. The vessel *A* has a capacity of about 50 cubic centimeters and carries a stopper with three perforations for the arrangement shown.

About six cubic centimeters of a concentrated aqueous solution of potassium nitrate are placed in *A* and the lower parts of the

⁷¹ Principles and Practice of Agricultural Analysis, 1908, 2 [2] : 367.

⁷² Landwirtschaftlichen Versuchs-Stationen, 1874, 17 : 321.

Zeitschrift für analytische Chemie, 1875, 14 : 380.

tubes *a* and *b* are filled with water to a little above *c* in order to exclude the air therefrom. Dilute sulfuric acid is placed in one of the funnels, and an aqueous solution of the amid in the other. The air is displaced from the empty part of *A* by introducing the sulfuric acid, a little at a time, whereby nitrous acid and nitric oxid are evolved. This operation is continued until all the air has been driven out through *c d*, the open end of *d* being kept in the liquid in the dish shown in Fig. 97. The

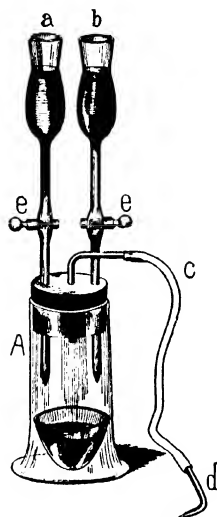


Fig. 96.—Apparatus for Amid Nitrogen.

eudiometer in which the evolved nitrogen is measured is shown in Fig. 97, and should have a capacity of about 50 cubic centimeters, and be graduated to fifths. It is filled with the solution of ferrous sulfate contained in *B* by sucking at *g*, after which the clamp *h* is replaced, the cock *f* closed, and the free end of *d* placed in the lower end of the eudiometer. The solution of the amid is run slowly into the generator *A*, Fig. 96, together with small additional quantities of the sulfuric acid when the evolution of gas becomes slow. From time to time *h* is opened and

fresh quantities of the ferrous solution allowed to flow into the eudiometer. Any trace of the amid remaining in the funnel is washed into *A* with pure water, with care to avoid the introduction of air. When the liquid in *A* assumes a permanent blue color the decomposition is complete. The residual gas is driven out of *A* by filling with water. The tubes *d* and *h*, after all the nitric oxid is absorbed, are removed from the eudiometer which is transferred to a cylinder containing water and immersed therein

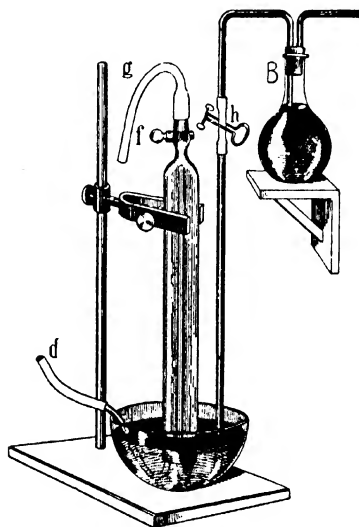


Fig. 97.—Sachsse's Eudiometer.

until the two liquid surfaces are at the same level and the volume of the nitrogen observed. After correction for temperature and pressure, the weight of the nitrogen is calculated. Twenty-eight parts by weight of nitrogen correspond to 150 of pure asparagin, 181 of tyrosin and 131 of leucin.⁷³ This method of procedure is difficult of manipulation and is apt to give results that are too high. It cannot be preferred to the more simple and accurate processes already described.

⁷³ Dragendorff, *Plant Analysis*, 1884, 245.

412. Preparation of Asparagin.—Asparagin is easily obtained from the aqueous extracts of plants by crystallization.⁷⁴ In case the analyst desires to prepare a quantity of asparagin for comparative purposes it may be easily accomplished in the following way: A sufficient quantity of peas or beans is sprouted in a dark place and allowed to grow until the reserve food of the seed is exhausted. The young sprouts are gathered, shredded and subjected to strong pressure. The juice thus obtained is boiled to coagulate the albumin, and thrown on a filter. The filtrate is evaporated to a thin sirup and set aside to allow the asparagin to separate in a crystallized form. If the crystals at first formed are colored they may be dissolved, decolorized with boneblack, and recrystallized. Instead of the above method the young shoots may be shredded, extracted with hot water and the extract treated as above. A larger yield of the asparagin is obtained by the latter process than by the one mentioned above.⁷⁵

413. Detection and Estimation of Asparagin and Glutamin. Of all the amid bodies asparagin is the most important from an agricultural standpoint, because of its wide distribution in vegetable products.⁷⁶ In addition to its crystalline characteristics asparagin may be identified by the following tests. Heated with alkalis, including barium hydroxid, asparagin yields ammonia. Boiled with dilute acids it forms ammonium salts. A warm aqueous solution dissolves freshly prepared copper hydroxid with the production of a deep blue color. Sometimes, on cooling, crystals of the copper compound formed are separated. Asparagin crystallizes with one molecule of water. Glutamin gives essentially the reactions characteristic of asparagin, but crystallizes without water in small white needles. Asparagin is easily detected with the aid of the microscope by placing sections of vegetable tissues containing it in alcohol. After some time microscopic crystals of asparagin are separated. The presence of large quantities of soluble carbohydrates seriously interferes with the separation of asparagin in crystalline form.

⁷⁴ *Landwirtschaftlichen Versuchs-Stationen*, 1873, **16** : 61.

⁷⁵ *Berichte der deutschen chemischen Gesellschaft*, 1877, **10** : 100, and 1883, **16** : 312. *Chemiker-Zeitung*, 1896, **20** : 145.

⁷⁶ *Zeitschrift für analytische Chemie*, 1883, **22** : 325.

For the detection of glutamin the liquid containing it is boiled with dilute hydrochloric acid, by which ammonia and glutamic acid are formed. On the addition of lead acetate to the solution the glutamic acid is thrown out as a lead salt, in which, after its decomposition with hydrogen sulfid, the characteristic properties of glutamic acid can be established.

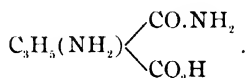
The above process is chronophagous and also uncertain where the quantity of glutamin is very small and that of other soluble organic matters very large. A much better process, both for the detection of glutamin and asparagin, is the following, based on the property possessed by mercuric nitrate of precipitating amids.

The aqueous extract containing the amid bodies is mixed with lead acetate until all precipitable matters are thrown out and the mixture poured into a filter. To the filtrate is added a moderately acid solution of mercuric nitrate. The precipitate produced is collected on a filter, washed, suspended in water, decomposed with hydrogen sulfid and again filtered. The amid bodies (glutamin, asparagin, etc.) are found in the filtrate and can be detected and estimated by the processes already described. A reaction showing the presence of an amid body is not a positive proof of the presence of asparagin or glutamin, since among other amids, allantoin may be present. This substance is found in the sprouts of young plants and also in certain cereals.⁷⁷ Allantoin, glutamin, and asparagin, when obtained in solution by the above process, may be secured, by careful evaporation and recrystallization, in well defined crystalline forms. Asparagin gives lustrous, rhombic prisms, easily soluble in hot water, but insoluble in alcohol and ether.

Allantoin is regarded as a diureid of glyoxalic acid and has the composition represented by the formula $C_4H_6N_4O_3$. It crystallizes in lustrous prisms having practically the same solubility as asparagin.

Glutamin is the amid of amidoglutamic acid. It crystallizes in fine needles. Its structural formula is represented as

⁷⁷ Richardson and Crampton, *Berichte der deutschen chemischen Gesellschaft*, 1886, **19** : 1180.



414. Cholin and Betain.—Cholin is a nitrogenous base found in both animal and plant tissues. Its name is derived from the circumstance that it was first discovered in the bile. It is found in the brain, yolk of eggs, hops, beets, cottonseed and many other bodies. When united with glycerophosphoric acid it forms lecithin, a compound of great physiological importance. From a chemical point of view, cholin is oxyethyltrimethyl-ammonium

hydroxid, $\text{C}_2\text{H}_4 \begin{cases} \text{OH} \\ \text{N}(\text{CH}_3)_3.\text{OH} \end{cases}$; $(\text{C}_3\text{H}_{15}\text{NO}_2)$. It is crystallized

with difficulty and is deliquescent. Its most important compound, from an analytical point of view, is its platinum salt $(\text{C}_5\text{H}_{14}\text{ONCl}_2)\text{PtCl}_4$. This salt crystallizes in red-yellow plates and is insoluble in alcohol.

Betain, $\text{C}_5\text{H}_{11}\text{NO}_2$, is the product of the oxidation of cholin.

The bases are separated from cottonseed and from each other by the process described below.⁷⁸

About five pounds of fine-ground cottonseed cake are extracted with 70 per cent. alcohol. The material should not be previously treated with dilute mineral acids because of the danger of converting a part of the cholin into betain. The alcohol is removed from the filtered extract and the residue dissolved in water. The aqueous solution is treated with lead acetate until no further precipitation takes place, thrown on a filter, the lead removed from the filtrate with hydrogen sulfid and the liquid evaporated to a viscous sirup. The sirup is extracted with alcohol containing one per cent. of hydrochloric acid. The solution thus obtained is placed in a deep beaker and the bases precipitated by means of an alcoholic solution of mercuric chlorid. The complete separation of the salts requires at least two weeks.

The double salts of the bases and mercury thus obtained, after freeing from the mother-liquor, are recrystallized from a solution in water and from the pure product thus obtained the mercury is

⁷⁸ Maxwell, American Chemical Journal, 1891, 13 : 470.

removed after solution in water, by hydrogen sulfid. The filtrate, after separating the mercury, contains the bases as chlorids (hydrochlorates). The solution of the chlorids is evaporated slowly in (pene) vacuo to a thick sirup and set over sulfuric acid to facilitate crystallization. The hydrochlorates are obtained in this way colorless and in well-shaped crystalline forms.

In a quantitative determination, a small amount of the fine meal is extracted at once with one per cent. hydrochloric acid in 70 per cent. alcohol, the salts obtained purified as above and weighed.

The following process serves to determine the relative proportions of cholin and betain in a mixture of the two bases.

A definite weight of the chlorids, prepared as directed above, is extracted by absolute alcohol. This treatment dissolves all the cholin chlorid and a little of the betain salt. The alcoholic solution is evaporated and again extracted with absolute alcohol. This process is repeated three times and at the end the cholin chlorid is obtained free of betain. In a sample of cottonseed cake the two bases were found present in the following relative proportions, *viz.*, cholin 17.5 per cent., betain 82.5 per cent. Thus purified the cholin is finally precipitated by platinum chlorid. For a description of the special reaction, by means of which cholin and betain are differentiated, the paper cited above may be consulted.

These bodies have acquired an economic interest on account of their occurrence in cottonseed meal, which is so extensively used as a cattle food. It is evident from the relative proportions in which they occur that the less nocuous base, betain, is the more abundant. It is possible, however, that the base originally formed is cholin and that betain is a secondary product.

Experience has shown that it is not safe to feed cottonseed meal to very young animals, while moderate rations thereof may be given to full-grown animals without much expectation of deleterious results. In the case of toxic effects it is fair to presume that a meal has been fed in which the cholin is relatively more abundant than the betain.

415. Lecithin.—Lecithin is a nitrogenous body, allied both to the fats and proteins and containing glycerol and phosphoric acid. Its percentage composition is represented with some accuracy by the formula $C_{42}H_{86}NPO_9$, or according to Hoppe-Seyler, $C_{44}H_{90}NPO_9$. It appears to be a compound of cholin with glycerolphosphoric acid. It is widely distributed both in animal and vegetable organisms, in the latter especially in peas and beans.

From a physiological point of view, lecithin is highly important as the medium for the passage of phosphorus from the organic to the inorganic state, and the reverse. This function of lecithin has been thoroughly investigated by Maxwell.⁷⁹

In the extraction of lecithin from seeds (peas, beans, etc.) it is not possible to secure the whole of the substance by treatment with ether alone.⁸⁰

The extraction of the lecithin may, however, be entirely accomplished by successive treatments for periods of about 15 hours with pure ether and alcohol. This is better than to mix the solvents, since, in this case, the ether having the lower boiling point is chiefly active in the extraction. When the extraction is accomplished by digestion and not in a continuous extracting apparatus the two solvents may be mixed together and thus used with advantage. After the evaporation of the solvents, the lecithin is ignited with mixed sodium and potassium carbonate whereby the organic phosphorus is secured without loss in an inorganic form. Where greater care is desired, the method described for organic phosphorus in soils may be used.⁸¹ The inorganic phosphorus thus obtained is estimated in the usual way as magnesium pyrophosphate.

For analytical purposes, the extraction of lecithin from vegetable substances is conducted as follows.⁸² The fine-ground pea or bean meal is placed in an extraction apparatus and treated continuously with anhydrous ether for 15 hours. The ether in the

⁷⁹ Maxwell, *American Chemical Journal*, 1893, **15** : 185.

⁸⁰ Maxwell, *American Chemical Journal*, 1891, **13** : 13.

Schulze, *Zeitschrift für physiologische Chemie*, 1889, **13** : 365.

⁸¹ *Principles and Practice of Agricultural Analysis*, 1906, **1** : 460.

⁸² Maxwell, *American Chemical Journal*, 1891, **13** : 15.

apparatus is replaced with absolute alcohol and the extraction continued for six hours longer. The alcoholic extract is evaporated to dryness and treated with ether. The part of the lecithin at first insoluble in ether becomes soluble therein after it has been removed from the vegetable tissues by alcohol. Moreover, any trace of inorganic phosphorus which may have been removed by the alcohol, is left undissolved on subsequent treatment with ether. The ether extract from the alcohol residue is added to that obtained directly, the ether removed by evaporation, and the total lecithin oxidized and the residue used for the estimation of phosphorus as already described.

In determining the lecithin in eggs, the procedure employed for vegetable tissues is slightly changed.⁸³ The whole egg, excluding the shell, is placed in a flask with a reflux condenser and boiled for six hours with absolute alcohol. The alcohol is then removed from the flask by evaporation and the residue treated in like manner with ether for 10 hours. The ether is removed and the dry residue rubbed to a fine powder, placed in an extractor and treated with pure ether for 10 hours. The extract thus secured is oxidized after the removal of the ether by fusion with mixed alkaline carbonates and the phosphorus determined in the usual way.

416. Factor for Calculating Results.—The percentage of lecithin is calculated from the weight of magnesium pyrophosphate obtained by multiplying it by the factor 7.2703.⁸⁴ This factor is calculated from the second formula for lecithin given above, in which the percentage of phosphorus pentoxid, P_2O_5 , is 8.789.

Example.—In 54 grams of egg, exclusive of the shell, is found an amount of organic phosphorus yielding 0.0848 gram of magnesium pyrophosphate. Then $0.0848 \times 7.2703 = 0.61652$ and $0.61652 \times 100 \div 54 = 1.14$. Therefore the percentage of lecithin in the egg is 1.14. While the above method has been used quite extensively in the study of lecithins, it is not regarded as

⁸³ Maxwell, *American Chemical Journal*, 1893, 15 : 188.

⁸⁴ Hoppe-Seyler, *Handbuch der physiologisch- und pathologisch-chemischen Analyse*,

satisfactory by analysts and indeed there is no method of lecithin determination that is.

417. Total Phosphorus in Lecithin.—The total phosphorus in lecithin may be estimated by any of the methods employed for the determination of phosphorus in organic combination. The purpose, of course, is to convert the whole of the phosphorus into phosphoric acid.

The method proposed by Freundler⁸⁵ suggests that the lecithin containing phosphorus be boiled with nitric acid, and the oxidation completed by the addition of potassium permanganate. In carrying out the process, about two grams of the lecithin are warmed with 50 cubic centimeters of fuming nitric acid at the temperature of boiling water for from two to three hours, until a pale yellow solution is obtained. After cooling, potassium permanganate is added in small quantities at a time until the permanganate color in the liquid is no longer discharged. Usually about 30 grams of the permanganate will be required. The mixture is diluted with water to 150 cubic centimeters, the precipitated manganese dioxid dissolved by sodium nitrite, and the solution evaporated to a sirupy consistence. This product is dissolved in about 70 cubic centimeters of water, the phosphoric acid precipitated with molybdic acid and determined in the usual manner.

418. Estimation of Alkaloidal Nitrogen.—The alkaloids contain nitrogen in a form more difficult of oxidation than that contained in protein or albuminoid forms. It is doubtful whether any of the nitrogen in alkaloids becomes available for plant nutrition by any of the usual processes of fermentation and decay to which nitrogenous bodies are submitted in the soil. Likewise, it is true that it is not attacked by the digestive processes in any way preparatory to its assimilation as food by the animal tissues. Alkaloidal nitrogen is therefore not to be regarded as a food either for the animal or plant.

For the general methods of estimating alkaloids the reader is referred to standard works on plant chemistry and toxicology. The alkaloids of interest in this manual are those which are found

⁸⁵ Bulletin de la Société Chimique, 1912, 11 : 1041.

in tobacco, tea, coffee and a few other products of agricultural importance. The best methods of isolating and estimating these bodies will be given in the part of the volume devoted to the special consideration of the articles mentioned.

SEPARATION OF PROTEIN BODIES IN VEGETABLE PRODUCTS.

419. Preliminary Treatment.—The chief disturbing components of vegetable tissues, in respect of their influence on the separation and estimation of the protein constituents, are fats and oils and coloring matters. In many cases these bodies are present in such small quantities as to be negligible, as, for instance, in rice. In other cases they exist in such large proportions as to present almost insuperable difficulties to analytical operations, as is the case with oily seeds. In all instances, however, it is best to remove these bodies, even when present in small proportions, provided it can be done without altering the character of the protein bodies. This is secured by extracting the fine-ground vegetable material first with petroleum ether, and afterwards with strong alcohol and ether. Practically all of the fatty bodies and the greater part of the most objectionable coloring matters are removed by this treatment. The extraction should in all cases be made at low temperatures, not exceeding 35° , to avoid the coagulating effect of higher temperatures upon the albuminous bodies which may be present.

Fatty seeds, as for instance peanuts, are first ground into coarse meal, then extracted with petroleum ether, ground to a fine meal and the fat extraction completed with petroleum ether, 95 per cent. alcohol and pure sulfuric ether. The residue of the last solvent may be removed by aspirating air through the extracted meal. In some cases, it is advisable to extract with ethyl ether before as well as after the alcoholic extraction. This treatment removes at least a part of the water and prevents the dilution of the first part of alcohol added to such an extent as to make it dissolve some of the protein matters. In each case, a portion of the alcoholic extract should be tested qualitatively for

protein matter. If any be found, stronger alcohol should be used for, at least, the first extraction. A portion of the meal, prepared as above directed, is extracted with a 10 per cent. solution of sodium chlorid, as described further on, and a measured portion of the filtered extract diluted with water until the protein matter in solution begins to be precipitated. By this treatment the proper strength of the salt solution, to be used for the subsequent extraction, is determined. To save time in dialyzing, the solution of salt employed as a solvent should be as dilute as possible.

The mixture of meal and solvent sometimes filters with difficulty. In these cases, it is advisable to first pour it into a linen bag from which the liquid portion can be removed by gentle pressure and subsequently filtered through paper. As a last resort, the liquid secured from the linen filter can be saturated with ammonium, zinc or magnesium sulfate, whereby all the protein matters are thrown out. After filtering, the residue is again dissolved in salt solution and can then be readily filtered through paper.

The clear filtrate should be tested by fractional precipitation by heat and the final filtrate by acetic acid, as will be described further on.

The protein matter may be further freed from amid compounds by treatment with copper sulfate.⁸⁶ This treatment is not advisable, however, except for the purpose of determining the total protein nitrogen in the sample. The action of the water, heat and cupric sulfate combined is capable of inducing grave changes in the character of the residual matter which would seriously interfere with the results of subsequent studies of the nature of the protein bodies.

In many instances, as with cereal grains, the separation of the protein bodies is accomplished by no further preliminary treatment than is necessary to reduce them to the proper degree of fineness.

420. Diversity of Character.—The proteins which occur in vegetable products are found in all parts of the tissues of the plants.

⁸⁶ Principles and Practice of Agricultural Analysis, 1908, 2 [2] : 557.

but in cereals especially in the seeds. In grass crops and in some of the legumes, such as clover, the nitrogenous matters are chiefly found in the straw and leaves. The general classification of these bodies has already been given, but each kind of plant presents marked variations, not only in the relative proportions of the different classes, but also in variations in the nature of each class. For this reason the study of vegetable proteins is, in some respects, a new research for each kind of plant examined. There are, however, some general principles which the analyst must follow in his work, and an attempt will be made here to establish these and to construct thereon a rational method of conducting the investigation. In the separation and estimation of complex bodies so nearly related to each other, it is difficult not only to secure satisfactory results, but also to prevent the transformation of some forms of protein matter into others nearly related thereto by the action of the solvents used for separation and precipitation.

421. Separation of Gluten from Wheat Flour.—The most important protein in wheat is the body known as gluten, a commercial name given to the nitrogenous matters insoluble in cold water. The gluten thus obtained does not represent a single chemical compound, but is a complex consisting of at least two protein bodies, which together form an elastic pasty mass, insoluble in cold water containing a trace of mineral salts. This mass has the property of holding mechanically entangled among its particles bubbles of gas, which, expanding under the action of heat during cooking, give to bread made of glutenous flours its porous property.

In respect of proteins, the American wheats, as a rule, are quite equal to those of foreign origin. This is an important characteristic when it is remembered that both the milling and food values of a wheat depend largely on the nitrogenous matter which is present. It must not be forgotten, however, that merely a high percentage of proteins is not always a sure indication of the milling value of a wheat. The percentage of gluten to the other protein constituents of a wheat is not always constant, and it is the gluten content of a flour on which its bread making qualities chiefly depend. The percentage of moist gluten gives,

in a rough way, the property of the glutenous matter of absorbing and holding water under conditions as nearly constant as can be obtained. In general, it may be said that the ratio between the moist gluten and the dry gluten in a given sample is an index for comparison with other substances in the same sample. Upon the whole, however, the percentage of dry gluten must be regarded as the safer index of quality. In respect of the content of glutenous matter, our domestic wheats are distinctly superior to those of foreign origin. They are even better than the Canadian wheats in this respect. It may be fairly inferred, therefore, that while our domestic wheats give a flour slightly inferior in nutritive properties to that derived from foreign samples, it is nevertheless better adapted for baking purposes, and this quality more than compensates for its slight deficiency in respect of nutrition, a deficiency which, however, is so minute as to be hardly worth considering.⁸⁷

The gluten is separated from the other constituents of a flour by the following process:

Ten grams of the fine-ground flour are placed in a porcelain dish, well wet with nearly an equal weight of water at a temperature of not to exceed 15°, and the mass worked into a ball with a spatula, taking care that none of it adheres to the walls of the dish. The ball of dough is allowed to stand for an hour, at the end of which time it is held in the hand and kneaded in a stream of cold water until the starch and soluble matter are removed. The ball of gluten thus obtained is placed in cold water and allowed to remain for an hour when it is removed, pressed as dry as possible between the hands, rolled into a ball, placed in a flat bottom dish and weighed. The weight obtained is entered as moist gluten. The dish containing the ball of gluten is dried for 20 hours in a steam-bath, again weighed, and the weight of material obtained entered as dry gluten. The determination of dry and moist gluten cannot in any sense be regarded as an isolation and estimation of a definite chemical compound. For millers' and bakers' purposes, however, the numbers thus obtained have a high practical value. A typical wheat grown in this country

⁸⁷ Division of Chemistry, Bulletin 45, 1895 : 51.

will contain about 26.50 per cent. of moist and 10.25 per cent. of dry gluten.

The gluten of wheat is composed of two protein bodies, gliadin and glutenin.⁸⁸ Gliadin contains 17.66 per cent. and glutenin 17.49 per cent. of nitrogen. Gliadin forms a sticky mass when mixed with water and is prevented from passing into solution by the small content of mineral salts present in the flour. It serves to bind together the other ingredients of the flour, thus rendering the dough tough and coherent. Glutenin serves to fix the gliadin and thus to make it firm and solid. Glutenin alone cannot yield gluten in the absence of gliadin, nor gliadin without the help of glutenin. Soluble metallic salts are also necessary to the formation of gluten, and act as suggested above, by preventing the solution of the gliadin in water, during the process of washing out the starch. No fermentation takes place in the formation of gluten from the ingredients named.

The gluten, which is obtained in an impure state by the process above described, is, therefore, not to be regarded as existing as such in the wheat kernel or flour made therefrom, but to arise by a union of its elements in the presence of water.

422. Extraction with Water.—It is quite impossible to get an extract from fine-ground vegetable matter in pure water because the soluble salts of the sample pass at once into solution and then a pure water solvent becomes an extremely dilute saline solution. The aqueous extract may, however, be subjected to dialysis, whereby the saline matter is removed and the protein matter, not precipitated during the dialytic process, may be regarded as that part of it in the original sample soluble in pure water. Nevertheless, in many instances, it is important to obtain an extract with cold water. In oatmeal the aqueous extract is obtained by Osborne as follows:⁸⁹ Five pounds of fine-ground meal are shaken occasionally with six liters of cold water for 24 hours, the liquid removed by filtration and pressure and the extraction continued with another equal portion of water in the manner noted. The two liquid extracts are united and saturated with

⁸⁸ Osborne and Voorhees, *American Chemical Journal*, 1893, 15 : 470.

⁸⁹ Osborne, *American Journal*, 1891, 13 : 385.

commercial ammonium sulfate which precipitates all the dissolved protein matter. The filtrate obtained is collected on a filter, washed with a saturated solution of ammonium sulfate and removed as completely as possible from the filter paper by means of a spatula. Any residual precipitate remaining on the paper is washed into the vessel containing the removed precipitate and the undissolved precipitate well beaten up in the liquid, which is placed in a dialyzer with a little thymol, to prevent fermentation, and subjected to dialysis for about two weeks. At the end of that time, the contents of the dialyzer are practically free of sulfates. The contents of the dialyzers are then thrown on a filter and in the filtrate are found those proteins first extracted with water, precipitated with ammonium sulfate and redissolved from this precipitated state by pure water. In the case of oatmeal, this protein matter is not coagulated by heat, and may be obtained in the dry state by the evaporation of the filtrate last mentioned at a low temperature in vacuo. It is evident that the character of the protein matter thus obtained will vary with the nature of the substance examined. In the case of oats, it appears to be a proteose and not an albumin.

423. Action of Water on Composition of Proteins.—When a body, such as oatmeal, containing many proteins of nearly related character, is exposed to the action of a large excess of water, the protein bodies may undergo important changes whereby their relations to solvents are changed. After oatmeal has been extracted with water, as described above, the protein matter originally soluble in dilute alcohol undergoes an alteration and assumes different properties. The same remark is applicable to the protein body soluble in dilute potash. Nearly all the protein matter of oatmeal is soluble in dilute potash, if this solvent be applied directly, but if the sample be previously treated with water or a 10 per cent. salt solution the subsequent proportion of protein matter soluble in dilute potash is greatly diminished.⁹⁰ Water applied directly to the oatmeal apparently dissolves an acid albumin, a globulin or globulins, and a proteose. The bodies, however, soluble in water exist only in small quantities in oat-

⁹⁰ Osborne, *American Chemical Journal*, 1891, 13 : 412.

meal. Experience has shown that in most instances, it is safer to begin the extraction of a cereal for protein matter with a dilute salt solution rather than with water, and to determine the matters soluble in water alone by subsequent dialysis.

424. Extraction with Dilute Salt Solution.—In general, it is advisable to begin the work of separating vegetable proteins by extracting the sample with a dilute brine usually of 10 per cent. strength. As conducted by Osborne and Voorhees, on wheat flour, the manipulation is carried on as follows:⁹¹

The fine-ground whole wheat flour, about four kilograms, is shaken with twice that weight of a 10 per cent. sodium chlorid solution, strained through a sieve, to break up lumps, and allowed to settle for 16 hours. At the end of this time, about half of the supernatant liquid is removed by a siphon or by decantation and filtered. Two liters more of the salt solution are added, the mixture well stirred and the whole brought onto the filter used above. The filtrate is collected in successive convenient portions and each portion, as soon as it is obtained, is saturated with ammonium sulfate. All the protein matter is precipitated by this reagent. The precipitate is collected on a filter, redissolved in a convenient quantity of the salt solution and dialyzed for 14 days or until all sulfates and chlorids are removed. The protein matter, which is separated on dialysis, in this instance, is a globulin. In the latest work Osborne recommends direct dialysis of the sodium chlorid extract without previous precipitation with ammonium sulfate.

The protein matter not precipitated on dialysis is assumed to be that part of the original substance soluble in water.

A part of the water soluble protein matter obtained as above is coagulated by heat at from 50° to 80°. The part not separated by heat gives a precipitate on saturation with sodium chlorid.

In wheat there are found soluble in water a coagulable albumins, leucosin and a proteose.⁹²

In separating the albumin coagulating at a low boiling point

⁹¹ Osborne, *Vegetable Proteins*, 1909 : 16.

⁹² Osborne, Report Carnegie Institution, 1907 : 100.

from the dialyzed solution mentioned above, it is heated to 60° for an hour, the precipitate collected on a gooch, washed with hot water (60°), and then successively with 95 per cent. alcohol, water free alcohol and ether. On drying the residual voluminous matter on the filter over sulfuric acid, it becomes dense and horny, having in an ash free state, according to Osborne, the following composition :

	Per cent.
Carbon	53.06
Hydrogen	6.82
Nitrogen	17.01
Sulfur	1.30
Oxygen	21.81

425. Treatment without Precipitation with Ammonium Sulfate.

—Where abundant means are at hand for dialyzing large volumes of solution, the preliminary treatment of the solution made with 10 per cent. sodium chlorid with ammonium sulfate may be omitted.

When the precipitated proteins are to be used for the estimation of the nitrogen therein contained, it has been proposed to substitute the corresponding zinc salt for the ammonium sulfate.⁹³ This reagent has given satisfactory results and while a larger experience is desirable before commending it as an acceptable substitute in all cases, yet its obvious advantage, in being free of nitrogen for the use mentioned, entitles it to careful consideration.

The manipulation, with the exception of the precipitation with ammonium sulfate, is the same as that described in the preceding paragraph. The globulins are completely precipitated when the dialysis is complete and may be separated from the soluble albumins and proteoses by filtration.

426. Separation of the Bodies Soluble in Water.—*Albumins.*—

By the methods of treatment just described, the protein matters soluble in 10 per cent. sodium chlorid solution are separated into two classes, *viz.*, globulins insoluble in pure water and albumins and proteoses soluble in pure water. The aqueous solution will also contain any amids or nitrogenous bases soluble in the dilute saline solution and in water. Osborne and Voorhees have found

⁹³ Zeitschrift für analytische Chemie, 1895, 34 : 562.

that the best way of separating the albumins in the pure aqueous solution is by the application of heat.⁹⁴ By means of a fractional coagulation the albumins are divided into classes, *viz.*, those separating at from 60° to 65° and those remaining in solution at that temperature but separating up to 85°. The respective quantities of these albumins are determined by collecting them in a filter and estimating the nitrogen therein by moist combustion in the usual way. Even a larger number of albumins may be secured, as in the maize kernel, by such a fractional precipitation by means of heat. Chittenden and Osborne find in this instance that the precipitation begins at about 40°.⁹⁵

Proteose.—After the separation of the albumins by heat the filtrate may still contain protein matter. This matter belongs to the proteose class. It may be partially secured by concentrating the filtrate, after the removal of the albumins, to a small bulk when a part of the proteose body will separate. It may be thrown out entirely by treating the filtrate above mentioned with fine-ground salt until it is saturated or by adding salt until the solution contains about 20 per cent. thereof and precipitating the proteose by acetic acid.⁹⁶

427. Separation of the Globulins.—The globulins which are extracted with 10 per cent. solution of sodium chlorid and precipitated on dialysis may be separated by fractional solution into several bodies of nearly related properties. This solution is conveniently accomplished by saline solvents of increasing strength. In the case of the maize globulins, Chittenden and Osborne employ dilute solutions of common salt for effecting the separation, beginning with a quarter of a per cent. and ending with a two per cent. mixture.⁹⁷

428. Proteins Soluble in Dilute Alcohol.—Some of the protein bodies which are soluble in dilute salt solution and in water are also soluble in alcohol. Since these bodies are more easily identified by the processes already described, attention will be given

⁹⁴ For latest procedure, see *Proteins of the wheat kernel* : 101.

⁹⁵ Chittenden and Osborne, *American Chemical Journal*, 1891, **13** : 455.

⁹⁶ Osborne and Voorhees, *American Chemical Journal*, 1893, **15** : 409.

⁹⁷ Chittenden and Osborne, *American Chemical Journal*, 1891, **13** : 464.

in this paragraph solely to those protein bodies which are insoluble in water or dilute salt solution and are soluble in dilute alcohol.

For the extraction of these bodies, the residue, left after extraction with a 10 per cent. solution of sodium chlorid or with water, is mixed with enough strong alcohol to secure by the admixture with the water present in the sample an alcohol of about 75 per cent. strength. The mixture is well shaken and digested for some time, at a temperature of about 46°, and thrown on a filter which is kept at about the same temperature. The residue is again mixed with alcohol of the same strength (75 per cent.) using about four liters for two and a half kilos of the original material. During the second digestion the temperature is kept at about 60°. The latter operation is repeated three times and in each case the filtrate obtained is evaporated separately.⁹⁸ This process is especially applicable to the meal from maize kernels, which contains a high relative percentage of an alcohol soluble protein, zein.

The chief part of the zein is found in the first two extracts, obtained as described above. On evaporation, the zein separates as a tough, leathery, yellow-colored mass on the walls of the containing vessel. It is cut into small pieces and digested for several days in cold, pure alcohol. This is followed by digestion with a mixture of ether and pure alcohol, and finally with pure ether. By this treatment a part of the zein becomes insoluble in 75 per cent. alcohol. The part soluble in dilute alcohol is precipitated by pouring it into water.

Another method of preparing zein is to extract the meal with 75 per cent. alcohol after it has been treated with a 10 per cent. salt solution.

In this case the extraction is continued with 75 per cent. alcohol in successive portions until no more protein matter passes into solution. The several extracts are united and the alcohol removed by distillation, by which process the zein is separated. It is washed with distilled water, until the sodium chlorid is removed, dissolved in warm alcohol of about 80 per cent. strength and

⁹⁸ Chittenden and Osborne, *American Chemical Journal*, 1892, 14: 32.

any insoluble matter removed by filtration. On evaporating the filtrate nearly to dryness, the zein is separated and pressed as free of water as possible, yielding a yellow, elastic substance resembling molasses candy. This preparation is purified by digestion with pure alcohol and ether in the manner described. The two zeins which are secured by the treatment, one soluble and the other insoluble in alcohol, are practically identical in composition.⁹⁹

Zein freshly precipitated by pouring its alcoholic solution in water is wholly insoluble in water, and, on boiling therewith, is changed into the variety insoluble in dilute alcohol. Boiled with dilute sulfuric acid, six in 300 cubic centimeters of water, it melts, forming a gummy mass, which is very slowly attacked by the acid yielding proteoses and peptones. Heated with stronger sulfuric acid it undergoes decomposition, yielding leucin, tyrosin, and glutamic acid.

429. Solvent Action of Acids and Alkalies.—In the preceding paragraphs, a synopsis has been given of the methods of separating protein matters in such a manner as to secure them in a pure state in the same conditions as they exist in the natural substances. A very large percentage of the protein matter is still left undissolved after extraction with the solvents already mentioned.

Often important information may be gained concerning the nature of the residual protein matters by fractional extraction with dilute acids and alkalies. When the strength of these solutions is such that they contain about one per cent. of the acid or alkali, the whole of the protein matter may be dissolved by boiling successively with acid and alkali for half an hour. The protein matter passing into solution in these cases is usually changed in character, assuming the nature of proteoses or allied bodies, when treated with an acid, and becoming albuminates when boiled with an alkali. Easily soluble carbohydrate matter is also removed by this treatment so that the residue obtained consists largely of cellulose and is known as crude or insoluble fiber. The removal of all the bodies soluble in dilute boiling acid

⁹⁹ Chittenden and Osborne, *American Chemical Journal*, , : .

and alkali is accomplished by the method described in paragraph 295.

For research purposes, the solvent action of dilute alkali is of chief importance to the analyst, and the extraction of the protein matter, after all that is soluble in water, common salt solution and alcohol has been removed, should commence with a solution of potassium or sodium hydroxid containing not over two-tenths per cent. of the alkali.

It has been shown by Osborne that the solvent action of very dilute alkali, in the cold, may be exerted without changing the character of the dissolved protein.¹

430. Method of Extraction.—The solvent employed is usually a two-tenths per cent. solution of potassium hydroxid. It may be added directly to the substance or may follow extraction with water, salt solution or alcohol. In the former case, the manipulation is illustrated by the following description of the treatment of oatmeal:²

One hundred grams of oatmeal are mixed with half a liter of a two-tenths per cent. potassium hydroxid solution and allowed to stand for some time at room temperature. The mixture is strained through a cloth to remove the chaff and the residue is stirred with another small portion of the solvent, again strained in the same cloth and the residue squeezed dry. The strained liquids are united and enough more of the solvent added to make the volume 700 cubic centimeters. After standing for some time, the insoluble matter settles to the bottom of the vessel and the supernatant liquid is decanted. More solvent is added to the residue, well mixed therewith and treated as above. It is advisable to make a third extraction in the same way. The extracts are united, passed through a filter, the protein matter in solution thrown out by acetic acid, washed with water, alcohol and ether and dried over sulfuric acid.

The methods of procedure, when the sample has been previously extracted with water, salt solution or alcohol, are essen-

¹ Osborne, *American Chemical Journal*, 1892, 14 : 639.

² Osborne, *American Chemical Journal*, 1891, 13 : 399.

tially the same as that just described and the reader may consult the papers of Osborne for details.³

431. Methods of Drying Separated Proteins.—In the preceding paragraphs, the analyst has been directed, in most instances, to dry the protein matter, after it is secured in as pure a form as possible, at room temperature, over sulfuric acid. By this treatment the preparation may be obtained in a form suited to the study of its physical properties, since its solubility has not been affected by subjecting it to a high temperature. When it is desired to use the sample only for chemical analysis it is not necessary to wait on the slow process above mentioned. In this case the sample may be dried in an inert atmosphere at the temperature of a steam-bath or even at 110° . It is better, however, to avoid so high a temperature and to conduct the desiccation in vacuo at a heat not above that of boiling water. The sample, before drying, should be reduced to the finest possible state of comminution, otherwise particles of aqueous vapor may be retained with great tenacity.

In many cases it is advisable to dry the sample pretty thoroughly, then grind to a fine powder and finish the desiccation with the pulverulent mass. This treatment can be followed when the quantity of the material is considerably in excess of that required for the analytical operations.

432. Determination of Ash.—No method of treatment is known by means of which vegetable protein matters may be obtained entirely free of mineral matters. The mineral bases may be naturally present in the protein matter as organic and inorganic salts, or they may be mechanically entangled therewith, having been derived either from the other tissues of the plant or from the solvents employed. It is necessary in calculating the analytical data to base the computation on the ash free substance. The percentage of ash is determined by any of the standard processes or by heating the sample in a combustion tube, to very low redness, in a current of oxygen. The total residue obtained is used in calculating the percentage of ash, and the weights of material

³ Osborne, *The Vegetable Proteins*, 1909 : 19, Proteins of the wheat kernel : 82.

subsequently used for the determination of carbon, hydrogen, nitrogen and sulfur are corrected for the calculations by deducting the quantity of mineral matter contained therein.

By reason of the highly hygroscopic nature of the dry protein bodies, they must be kept over a desiccating material and weighed quickly on a balance, in an atmosphere which is kept free of moisture by the usual methods.

433. Carbon and Hydrogen.—Carbon and hydrogen are estimated in protein matters by combustion with copper oxid. Osborne prefers to burn the sample in a platinum boat in a current of air or of oxygen free of moisture and carbon dioxide.⁴ It is advisable to use also a layer of lead chromate in addition to the copper oxid and metallic copper. The method of conducting the combustion has already been described.⁵ The analyst should have at his disposal a quantity of pure sugar, which may be used from time to time in testing the accuracy of the work. In beginning a series of combustions this precaution should never be omitted. The addition of the lead chromate is to make more certain the absorption of oxidized sulfur produced during the combustion.

434. Estimation of Nitrogen.—In most cases it is found convenient, during the progress of separating vegetable proteins, to determine the quantity of each kind by estimating the nitrogen by moist combustion and computing the quantity of protein matter by multiplying the nitrogen by 6.25. The estimation of the nitrogen is made either on an aliquot part of the extract or by direct treatment of the residue.

In the pure extracted protein matter the nitrogen is most conveniently determined by moist combustion, but it may also be obtained either by combustion with soda-lime or with copper oxid, or by other reliable methods.⁶

The percentages of nitrogen found in the principal protein bodies, together with the factors for computing the weights of

⁴ Osborne, *American Chemical Journal*, 1891, **13** : 409.

⁵ *Principles and Practice Agricultural Analysis*, 1906, **1** [2] : 330.

⁶ *Principles and Practice Agricultural Analysis*, 1908, **2** [2] : 321 et seq.

the protein bodies from the weights of nitrogen found, are given below.⁷

Name of body.	Percentage of nitrogen.	Factors.
Mucin	10.80-14.91	9.26-6.71
Prot. albumin	13.46-14.98	7.43-6.70
Serum globulin	15.85	6.31
Egg albumin	15.00-15.51	6.67-6.45
Maize Zein	16.13	6.20
Casein	14.65-16.44	6.83-6.14
Serum-albumin	15.91	6.29
Keratin	15.71-17.9	6.37-5.59
Fibrinogen	16.66	6.00
Elastin	14.67-17.74	6.82-5.64
Wheat proteins	16.93-17.66	5.91-5.64
Fibrin	16.91	5.91
Flax seed globulin	18.48	5.41

435. Determination of Sulfur.—Sulfur is a characteristic constituent of the protein bodies, existing in quantities approximating one per cent. of their weight.

In the estimation of sulfur, it is first converted into sulfuric acid, which is thrown out by a soluble barium salt and the sulfur finally weighed as barium sulfate.

All the sulfur existing in the organic state in a protein may be obtained by burning in a current of oxygen and conducting the gaseous products of combustion through solid sodium or potassium carbonate at or near a red heat.⁸ The organic sulfur may also be converted into sulfuric acid by fusing the protein body with a mixture of sodium hydroxid and potassium nitrate. The fused mass, after cooling, is dissolved in water, the solution acidified with hydrochloric, evaporated to dryness to decompose nitrates and remove excess of hydrochloric acid and dissolved in a large excess of water. After standing for a day, the solution is filtered and the sulfuric acid thrown out of the hot filtrate with a slight excess of barium chlorid solution. The usual pre-

⁷ *Aberhalden, Biochemischer Handlexicon*, 4, Part I : 43, 58, 66, 75, 82, 96, 103, 140, 185, 194.

Osborne, Vegetable Proteins : 57.

⁸ *Principles and Practice Agricultural Analysis*, 1906, 1 [2] : 470.

cautions in precipitating, filtering and igniting the barium sulfate are to be observed.⁹

436. General Observations.—In the preceding paragraphs have been stated the general principles upon which the separation of vegetable protein matters depends, and a description has been given of the several processes by which this separation is accomplished. In each case, however, special conditions exist which require special modifications of the general processes, and these can only be successfully secured by the skill, judgment and patient labor of the investigator. Many of these cases have been already worked out, and the valuable data secured by Chittenden, Osborne and others are accessible to the analyst in the papers already cited. It is only by a careful study of the work already done as outlined here and as published in full in the cited papers, that the analyst will be able to secure trustworthy guidance for future investigations.

437. Dialysis.—One of the most important of the operations connected with the separation and analysis of proteins is the removal of the salts whereby their solutions are secured. This is accomplished by subjecting the solutions of the protein matters to dialysis. The solution is placed in bags made of parchment dialysis paper. These bags are tied about a glass tube, whereby access may be had to their contents during the progress of the work. Since the volume of the liquid increases during the process, the bags should not be filled too full in the beginning.

The dialysis may be carried out with the city water supply, which is first passed through a battery of porous porcelain filtering tubes to remove any suspended silt or micro-organisms. The water used should be as free from mineral matter as possible. If unfiltered water be used, the germs therein cause a fermentation in the protein matter, which seriously interferes with the value of the data obtained, and which can only be avoided by the use of an antiseptic, such as an alcoholic solution of thymol. Even with filtered water, the use of a few drops of the solution mentioned is often necessary. To avoid the use of too great quantities of

⁹ Osborne, *American Chemical Journal*, 1891, **13** : 410.

Bureau of Chemistry, *Bulletin*, 107 (revised) 1912 : 23.

the filtered water, the dialyzers are arranged *en batterie*, as shown in the figure. The filtered water enters the first vessel and thence passes through all. The parchment bags are frequently changed from vessel to vessel, each being brought successively into the first vessel in contact with the fresh water. In some cases the final steps in the dialysis may be accomplished in distilled water.

It is advisable to conduct a fractional preliminary dialysis of the salt solution containing proteins in such a way as to secure

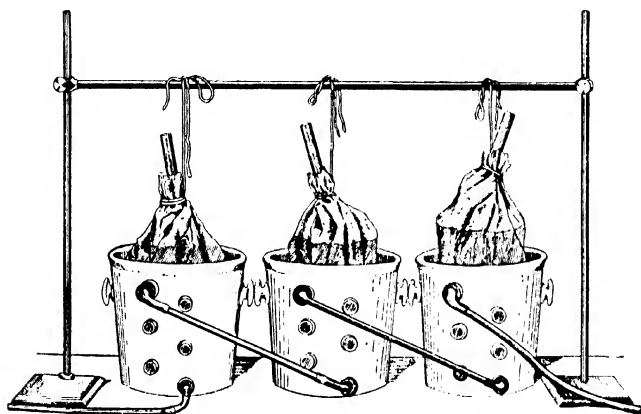


Fig. 98.—Dialyzing Apparatus.

the globulins precipitated in each interval of 24 hours. Each portion thus secured may be examined with the microscope. Usually a period of two weeks is required to entirely remove the mineral salts from solution. If prepared parchment tubes be used for the dialysis, they should be first tested for leaks, and should not be more than half filled. By the use of a large number of these tubes a greater surface is exposed to dialytic action, and the time required to complete the operation is correspondingly decreased.

SEPARATION AND ESTIMATION OF NITROGENOUS BODIES IN ANIMAL PRODUCTS.

438. Preparation of the Sample.—Animal products present many difficulties in respect of the reduction thereof to a sufficiently comminuted condition for analytical examination. In the case of bones, the choppers used for preparing them for feeding to fowls are the most efficient apparatus for reducing them to fragments. In this condition they may be ground to a finer state in a sausage machine. The flesh of animals may be reduced by this machine, with two or three grindings, to a fairly homogeneous mass. Subsequent grinding in a mortar with powdered glass or sharp sand may serve to reduce the sample to a finer pulp, but is not usually necessary and should be avoided when possible. The sample thus prepared serves for the estimation of water, ash and fat by methods already described. The sample should be prepared in quantities of considerable magnitude, the whole of any organ or separate portion of the body being used when possible. In examining the whole body the relative weights of blood, bones, viscera, muscle, hide and other parts should first of all be ascertained and noted.

439. Treatment of Muscular Tissues for Nitrogenous Bodies.—For the present purpose a brief sketch of the method of separating the nitrogenous bodies in the muscular tissues of the body is all that can be attempted. For methods of examining the different organs and parts of the body in greater detail, standard works on physiological chemistry may be consulted.¹⁰

Extraction with Cold Water.—A noted quantity of the finely divided tissues is mixed with several volumes of ice cold water and well rubbed occasionally for several hours, the temperature meanwhile being kept low. The mixture is poured into a linen bag and the liquid portion removed by gentle pressure. The residue in like manner is treated with fresh portions of cold water until it gives up no further soluble matters. An aliquot portion of the extract is concentrated to a small bulk and serves for the determination of total nitrogen. The methods of separating and

¹⁰ Hoppe-Seyler, *Handbuch der physiologisch- und pathologisch-chemischen Analyse*, 8th Edition, 1909.

estimating nitrogenous bodies in flesh soluble in water will be given in considerable detail further on.

Extraction with Ammonium Chlorid and Hydrochloric Acid.—The residue, after exhaustion with cold water, is extracted with a solution of ammonium chlorid containing 150 grams of the salt in a liter. This method of extraction is entirely similar to that with water just described. Globulins and myosin pass into solution by this treatment. The residual mass is washed as free as possible of the solvent and is then further extracted with dilute hydrochloric acid containing four cubic centimeters of the fuming acid in a liter. The treatment with dilute acid is continued until no further substance passes into solution. This is determined by neutralizing a portion of the extract with sodium carbonate, or by the direct addition of potassium ferrocyanid. In either case absence of a precipitate indicates that no nitrogenous matters are present in the solution.

Extraction with Alkali.—The residue from the acid extraction is washed with water until the acid is removed and then extracted in a similar manner with a dilute solution of sodium or potassium hydroxid containing not to exceed two grams of the caustic to the liter. When this residue is finally washed with water and a little acetic acid, it will be found that practically all the purely albuminous bodies contained in the tissues have been extracted with the exception of any fibrin, which the blood, present in the tissues at the commencement of the extraction, may have contained. The extract should be acidified with acetic as soon as obtained.

Extraction with Boiling Water.—The residual matter boiled for some time with water will part with its collagen, which, when transformed by the heat into gluten, passes into solution.

The sarcolemma, membranes, elastic fibers and keratin remain undissolved.

440. Contents of the Several Extracts.—By the systematic treatment of muscular tissue in the manner just described, the nitrogenous bodies they contain are separated into five classes, *vis.*:

Cold Water Extract.—This contains serum albumin, serum globulin, muscle albumin, myosin, mucin and peptone.

Ammonium Chlorid Extract.—This solution contains the globulins and also in many cases some myosin and serum globulin.

Hydrochloric Acid Extract.—When the extractive matter removed by hydrochloric acid, thrown out by sodium carbonate and well washed with water, has a neutral reaction, it consists of syntonin, when acid, of an albuminate.

Alkali Extract.—The acid albumin of the animal tissue is found in the alkaline solution and may be thrown out by making the solution slightly acid.

Insoluble Residue.—The fifth class contains the insoluble nitrogenous bodies mentioned above.

441. General Observations.—Only a brief résumé of the methods of treating animal tissues for nitrogenous bases is given above, since a more elaborate discussion of these principles and methods would lead too far away from the main purpose of this manual. For practical purposes, the most important of these bodies are those soluble in water and the methods of treating these will be handled at some length. Unfortunately, the methods of determining the exact qualities of these bodies are not as satisfactory in case of animal as in vegetable nitrogenous bodies. The flesh bases, soluble in water, contain a much larger percentage of nitrogen than is found in true protein bodies, and therefore the multiplication of the weight of nitrogen found therein by 6.25 does not give even a near approximation of the actual quantities of the nitrogenous bodies present in the sample.

Some of the flesh bases contain more than twice as much nitrogen as is found in proteins, and in such cases 3.12, and not 6.25, would be the more correct factor to use in the computation. When possible, therefore, these bodies should be precipitated and weighed after drying, but this is not practicable in many instances. The sole resource of the chemist in such cases is to determine

the nature of the body as nearly as possible by qualitative reactions, then to determine the total nitrogen therein and multiply its weight by the corresponding factor. The principal flesh bases have the following percentages of nitrogen and the approximate factors for calculating analytical data are also given:

Name of base	Formula	Per cent, nitrogen	Factor
Carnin.....	$C_7H_8N_4O_3$	28.62	3.48
Kreatin.....	$C_4H_9N_3O_2$	32.06	3.12
Kreatinin.....	$C_4H_7N_3O$	37.17	2.69
Sarkin.....	$C_3H_4N_4O$	41.17	2.43

442. Composition of Meat Extracts.—The meat extracts of commerce contain all the constituents of meat that are soluble in warm water. The parts which are soluble in warm water and not in cold are found in the cold aqueous solution as suspended or sedimentary matters. Among the nitrogenous bodies present are included albumin, albumose and peptone among the proteins, carnin, kreatin, kreatinin, sarkin and xanthin among the non-proteins, and inosinic and uric acids and urea among other nitrogenous bodies. Among the non-nitrogenous bodies are found lactic and butyric acids, inosit and glycogen. Among mineral bodies occurs the phosphates and chlorids of the common bases. In addition to these bodies, meat extracts may also contain gelatin and other decomposition products of protein matter. Since meat extract is supposed to be prepared by the digestion of the meat free of bones and put in cold water or in warm water not above 75°, the presence of gelatin would indicate a different method of preparation, *viz.*, either by boiling water or water heated above the boiling point under pressure. In a properly prepared extract, the percentage of gelatin is very small.

Approximately one-tenth of the whole nitrogen present is in the form of albumoses and only a trace as peptones. By far the greater part of the nitrogen exists as flesh bases (kreatin, etc.). The composition of three meat extracts, numbers one and two solid and number three liquid, is given in the subjoined table.¹¹

¹¹ Bureau of Chemistry, Bulletin, 114, 1908: 16-17.

	No. 1. Per cent.	No. 2. Per cent.	No. 3. Per cent.
Total nitrogen.....	9.28	9.14	2.77
Nitrogen as albumin.....	trace	0.68	trace
“ “ albumose.....	0.96	1.21	0.70
“ “ peptone.....	trace	trace	none
“ “ flesh bases.....	6.81	5.97	1.56
“ “ ammonia.....	0.47	0.41	0.69
“ in compounds insoluble in 66 per cent. alcohol.....	0.21	0.33	0.25
“ “ other bodies.....	0.83	1.14	0.17

443. Analysis of Meat Extracts.—The analysis of a meat extract should include the determination of the water, ash and total nitrogen. After multiplying the nitrogen which exists as proteins by 6.25 and as flesh bases by 3.12 and adding together the percentages of all the ingredients, ash, water, etc., including ammonia, the sum is to be subtracted from 100 and the difference entered as non-nitrogenous organic matter. The nature of this conglomerate has already been explained.

Water.—It is advisable to determine the water in a partial vacuum (18-21) or in an atmosphere of hydrogen (22-24).

The water may also be determined in solid extracts by placing about five grams of the material in a flat bottom tinfoil dish about 55 millimeters in diameter and 20 millimeters deep. The material is dissolved in enough warm water to fill the dish a little over one-half and the liquid is then absorbed by adding a weighed quantity of fibrous asbestos or of dry fragments of pumice stone. The asbestos is to be preferred because of the fact that it may be subsequently cut into small bits for the determination of the gelatin. The dish thus prepared is dried to constant weight in a steam-bath or vacuum oven. The weight of the dish and of the added absorbent, together with that of the material employed and of the dried dish and its contents, give the data for calculating the percentage of water. The contents of the dish are used as described further on for the determination of gelatin. In liquid extracts the water is determined in an entirely analogous manner, using about 20 grams of the material and omitting the solution in water.

In solid extracts, the part insoluble in cold water is determined separately.

Ash.—The ash is determined by ignition at the lowest possible temperature, best in a muffle (36-45). The ash should be examined qualitatively. Where a quantitative analysis is desired, larger quantities of the extract are incinerated and the constituents of the ash determined in the usual way.¹²

Total Nitrogen.—Since nitrates are not present unless added in the manufacture, the total nitrogen is best determined by moist combustion.¹³

Nitric Nitrogen.—The extract should be tested for nitrates and if present they are determined in the manner already described.¹⁴

Ammoniacal Nitrogen.—When ammonia is present it is determined by distillation with magnesia.¹⁵

Since boiling with magnesia may cause the distillation of more ammonia than is present as ammonium salts, the plus being due to the decomposition of some other nitrogenous compounds, Stutzer replaces the magnesia with barium carbonate.¹⁶

Protein Nitrogen Insoluble in 62 Per Cent. Alcohol.—The aqueous solution is treated with strong alcohol until the mixture contains about 62 per cent. of the reagent. The precipitate produced is separated by filtration, washed with 62 per cent. alcohol and the nitrogen therein determined.

Albumose (Protease) Nitrogen.—This is secured by saturating the aqueous solution with zinc or ammonium sulfate. The separated albumoses are skimmed from the surface, thrown on a filter, washed with a saturated solution of zinc sulfate and the nitrogen determined therein by moist combustion. In the filtrate from the above separation, peptone is detected qualitatively by

¹² Principles and Practice Agricultural Analysis, 1908, 2 [2] : 526 et seq. Bureau of Chemistry, Bulletin 107 (revised) 1912 : 21.

¹³ Bureau of Chemistry, Bulletin 107 (revised) 1912 : 5.

¹⁴ Bureau of Chemistry, Bulletin 107 (revised) 1912 : 10.

¹⁵ Principles and Practice Agricultural Analysis, 1908, 1 [2] : 503; 1906, 2 [2] : 388.

Bureau of Chemistry, Bulletin 107 (revised) 1912 : 10.

¹⁶ Zeitschrift für analytische Chemie 1895, 34 : 377.

adding a few drops of dilute solution of copper sulfate (biuret reaction).

Kreatin, Kreatinin and Other Flesh Bases.—The clear, aqueous solution of the extract is acidified with sulfuric, mixed with a solution of sodium phosphotungstate and allowed to stand for about six days. The precipitate is collected, washed with a solution of the precipitant, and the nitrogen therein determined. The nitrogen found, less that due to ammonia, represents the total nitrogenous matter precipitated by the phosphotungstic acid. From this quantity is deducted the nitrogen in the proteins, precipitated by 62 per cent. alcohol and by ammonium or zinc sulfate, and the remainder represents the nitrogen in flesh bases.

The nitrogen thrown out by the phosphotungstic acid is deducted from the total nitrogen, and the remainder represents the nitrogenous bodies not precipitable by the reagent named.

This method of separating the nitrogenous matters in meat extracts is based on the observation that these bodies contain at most only a small quantity of peptones, so small as to be safely negligible.¹⁷

Quantities Used for Analysis.—In conducting the separations above noted, it will be found convenient to use in each case about five grams of the solid or 20 of the liquid extract. In the nitrogen determinations, the weight of the sample should be inversely proportional to its content of nitrogen.

444. Preparation of the Phosphotungstic Reagent.—The phosphotungstic reagent is conveniently prepared as follows:

Dissolve 120 grams of sodium phosphate and 200 of sodium tungstate in one liter of water and add to the solution 100 cubic centimeters of strong sulfuric acid. When the reagent is prepared for general purposes it is customary to acidify with nitric, but in the present instance, inasmuch as the precipitate is used for the determination of nitrogen, it is evident that sulfuric should be substituted for nitric acid. In all cases the analyst must be assured of the strong acidity of the reagent, and in addition to this the solutions of protein matter to which the reagent is added must first be made strongly acid with sulfuric.

¹⁷ König and Bömer, *Zeitschrift für analytische Chemie*, 1895, 34 : 560.

445. Zinc Sulfate as Reagent for Separating Albumoses from Peptones.—When the albumoses (proteoses) are separated from the peptones, by precipitation with ammonium sulfate, there may be danger of some of this reagent adhering to the albumose, and in this way the quantity of nitrogen obtained on analysis may be increased. To avoid an accident of this kind Bömer replaces the ammonium by zinc sulfate.¹⁸

Since the precipitation of the albumoses by saturated saline solutions depends on their hygroscopic power, the substitution of another salt for ammonium sulfate capable of strongly attracting water, may be made if that salt does not possess any objectionable property. Crystallized zinc sulfate will dissolve in less than its own weight of cold water and is therefore well suited for the purpose in view.

In the case of a meat extract, the precipitation is accomplished as follows: Fifty cubic centimeters of the extract, freed from all solid matter by filtration and containing about two grams of the soluble proteins, are saturated in the cold with finely powdered zinc sulfate. The separated albumoses collect on the surface and are skimmed off, poured on a filter and washed with cold saturated zinc sulfate solution. The filter and its contents are used for the determination of nitrogen by moist combustion.¹⁹

The filtrate from the precipitated albumoses gives no biuret reaction and, therefore, as in the use of ammonium sulfate, is free of albumin.

The biuret reaction is applied to the zinc sulfate filtrate as follows: The filtrate is greatly diluted with water and freed of zinc by means of a saturated solution of sodium carbonate. The filtrate free of zinc is evaporated on the steam-bath, made strongly alkaline with sodium hydroxid and treated with a few drops of a two per cent. copper sulfate solution, added successively.

Another advantage possessed by the zinc sulfate is found in

¹⁸ König and Bömer, *Zeitschrift für analytische Chemie*, 1895, **34** : 562.

¹⁹ *Principles and Practice of Agricultural Analysis*, 1908, **2** [2] : 347 et seq.

Bureau of Chemistry, Bulletin 107 (revised), 1912 : 5 et seq.

the fact that in the filtrate from the separated albumoses the peptones and other flesh bases can be thrown out by phosphotungstic acid. Before the application of the reagent, the filtrate should be made strongly acid by adding about an equal volume of dilute sulfuric acid (one part of acid to four of water).

The nitrogen in the precipitate thus obtained is determined by moist combustion in the manner already suggested.

If the protein matters contain salts of ammonium it is probable that a difficultly soluble double sulfate of zinc and ammonium, $(\text{NH}_4)_2\text{SO}_4 \cdot \text{ZnSO}_4 \cdot 6\text{H}_2\text{O}$, will be found in the precipitate. Ammonium salts, if present, should therefore be removed by distillation with magnesia. It is better, however, to throw down the ammonia with the first zinc precipitate, distil this with magnesia and determine the amount of nitrogen derived from the ammonia compounds. In a second sample, the total nitrogen is determined by moist combustion and the difference between the two results gives that due to albumoses.

446. Examination for Muscular Tissue.—Some samples of meat extracts contain small quantities of finely ground muscular tissue. For detecting this the extract is treated with cold water and the insoluble residue examined with a microscope. If muscular tissue be found, about eight grams of the solid extract or 25 of the fluid preparation are treated with cold water, the insoluble matter collected upon a filter, washed with cold water, and the nitrogen determined in the residue. The percentage of nitrogen multiplied by 6.25 gives the quantity of muscle fiber protein present. The filtrate from the above determination is acidified with acetic, boiled, any precipitate which is formed collected and the nitrogen therein determined. The nitrogen obtained multiplied by 6.25 gives the quantity of coagulable albumin present. An aliquot portion of the filtrate is used for the determination of nitrogen and the percentage therein found, deducted from the total nitrogen of the sample, gives a remainder which may be used as a representative of the whole of the nitrogen present in the form of albumin and muscular tissue.

447. Estimation of Gelatin.—The tinfoil dish and its contents used for the determination of water, as above described, are cut

into small pieces, placed in a beaker and extracted four times with absolute alcohol. After the removal of the alcohol, the residue is extracted with ice water containing 10 per cent. of alcohol, in which a small piece of ice is kept to avoid a rise of temperature. The beaker should be shaken during the extraction, which should last for about two minutes. Where large numbers of samples are treated at once, any convenient form of shaking machine may be employed. At least two extractions with ice water must be made. The residue is then collected upon a filter and washed with ice water until the washings are completely colorless. The residue on the filter is replaced in the beaker, boiled with water, well washed on the filter with boiling water, the filtrate and washings concentrated and the nitrogen therein determined.

The principle of this determination is based on the fact that gelatin is almost completely insoluble in ice water while serum peptones and albumin peptones are almost completely soluble in that reagent. On the other hand, the flesh bases and the proteins present are almost completely removed by the preliminary treatment with alcohol and ice water or are left undissolved by the hot water. The solution in boiling water, therefore, contains practically nothing but gelatin.²⁰

In a later article, Stutzer modifies the method given above as follows:²¹

Of dry and moist extracts from five to seven grams and of liquid extracts from 20 to 25 grams are used for the determination and placed in tinfoil dishes, as described above. In case of solid extracts, a sufficient quantity of warm water is added to completely dissolve them, the solution being facilitated by stirring. In case the solution is too thin it should be concentrated before going further. It is treated with a sufficient amount of dust-free ignited sand to completely absorb it, and the dish and its contents are then dried to a constant weight. The dried contents of the dish are rubbed up in a mortar, the dish cut into

²⁰ *Zeitschrift für analytische Chemie*, 1895, **34** : 374.

²¹ *Zeitschrift für analytische Chemie*, 1895, **34** : 568.

fine bits, and all placed in a beaker. The solid syrphete²² is extracted four times with 100 cubic centimeters of absolute alcohol, the alcohol in each case being poured through an asbestos filter for the purpose of collecting any matters suspended therein. In a large flask are placed 100 grams of alcohol, 300 grams of ice and 600 grams of cold water, and the flask is placed in a large vessel and packed with finely divided ice. Four beakers marked *b*, *c*, *d*, *e* are also placed in ice and the beaker containing the syrphete, left after extraction with absolute alcohol as above mentioned, is marked *a* and also placed in pounded ice. The extraction with cold alcoholic water proceeds as follows:

In beaker *a* are poured 100 cubic centimeters of the mixture in the large flask, its contents are stirred for two minutes and then the liquid portion poured off into beaker *b* to which, at the same time, a piece of ice is added. In beaker *a* are poured again 100 cubic centimeters from the large flask, treated as above described, and the liquid extract poured into beaker *c*. In like manner the extraction in beaker *a* is continued until each of the beakers has received its portion of the extract. By this time the liquid over the sand in beaker *a* should be completely colorless. The filtration of the liquid extract is accomplished as follows:

In a funnel of about seven centimeters diameter is placed a perforated porcelain plate about four centimeters in diameter which is covered with asbestos felt with long fiber. Three filters are prepared in this way. On the first filter are poured the contents of beaker *b*. After the liquid has passed through, the sand and other residue in beaker *a* are transferred to the filter and the beaker and residue washed with the alcoholic ice water from the large flask. The filtration should be accomplished under pressure. On the second filter are poured the contents of beaker *c*. On the third filter the contents of beakers *d* and *e*. The washing with alcoholic ice water from the large flask is continued in each instance until the filtrate is colorless. At the same time the asbestos filter, which was used in the first instance for filtering the absolute alcohol extract, is washed with the alcoholic ice water mixture from the large flask. At the end the sand remaining in

²² From *συρφετος*.

beaker *a* together with all the asbestos filters are brought together into a porcelain dish, boiled two or three times with water, the aqueous solution filtered and the filtrate concentrated and used for the estimation of the nitrogen. The quantity of nitrogen found multiplied by 6.25 represents the protein matter in the gelatin of the sample.

The object of the multiple filters, described above, is to accelerate the process, and they are required because the gelatin quickly occludes the filter pores. For this reason the asbestos filters are found to operate better than those made of paper. It should be mentioned that the residue of the peptones insoluble in alcohol may contain, in addition to gelatin, also small quantities of albumoses. From the quantity of albumose nitrogen found, it is understood that the nitrogen in the form of coagulable albumin, determined as described in the first process mentioned above, is to be deducted, since these coagulable albumins are insoluble in alcohol.

448. Estimation of Nitrogen in the Flesh Bases Soluble in Alcohol.—About five grams of the dry extract, 10 grams of the extract containing water or 25 grams of the liquid extract are placed in a beaker and enough water added in each case to make about 25 cubic centimeters in all. Usually no water need be added to the liquid extracts. Very thin peptone solutions should be evaporated until the content of water is reduced to 75 per cent. The solution, prepared as above indicated, is treated slowly with constant stirring with 250 cubic centimeters of absolute alcohol, the stirring continued for some minutes and the vessel set aside for 12 hours, at the end of which time the precipitate is separated by filtration and washed repeatedly with strong alcohol. Leucin, tyrosin and a part of the flesh bases are dissolved by alcohol. The alcohol is removed by distillation and the residue dissolved in water. Any flocky residue which remains on solution with water is removed by filtration, the nitrogen determined therein and the quantity thereof added to the albumose nitrogen found, as hereafter described.

The volume of the aqueous solution is completed with water to half a liter. One hundred cubic centimeters of this solution are

used for the determination of total nitrogen, and another 100 cubic centimeters for the determination of ammoniacal nitrogen by distillation with barium carbonate. A part of the ammonia may have escaped during the preliminary distillation of the alcohol and therefore the amount found may not represent the whole amount originally present. The purpose of the above determination is principally to ascertain the correction to be made in the amount of total nitrogen found in the first 100 cubic centimeters of the solution.

449. Treatment of the Residue Insoluble in Alcohol.—The residue insoluble in alcohol is washed from the filter into the beaker in which the first solution was made. The aqueous mixture is warmed on a water-bath until the alcohol adhering to the precipitate is completely evaporated, when the contents of the beaker are poured upon a filter free of nitrogen. A small part of the albumose, by reason of the treatment with alcohol, tends to remain undissolved, and it is advisable to collect this albumose upon a filter, wash it well with hot water and estimate the nitrogen therein. The quantity of nitrogen thus found is to be added to the albumose nitrogen determined as described later on.

The total filtrate obtained from the last filtration is made up to a volume of half a liter, of which 50 cubic centimeters are used for the determination of total nitrogen, 50 cubic centimeters for the determination of gelatin, albumose and peptone, and 100 cubic centimeters for the residual peptones. The albumose, together with the gelatin and peptones carried down with it, is precipitated with zinc or ammonium sulfate solution, and its per cent. calculated from the amount of nitrogen found in the precipitate. The true peptone is determined by subtracting the quantity of nitrogen determined as albumose from the total nitrogen in solution.

The rest of the liquid, *viz.*, 300 cubic centimeters, is evaporated to a small volume and tested qualitatively for true peptones as follows:

To separate the albumose and gelatin the concentrated liquor is treated with an excess of finely divided ammonium sulfate so that a part of the salt remains undissolved. The separated albumose, gelatin and undissolved ammonium salts are collected on a

filter, the filtrate mixed with a few drops of dilute copper sulfate solution and a considerable quantity of concentrated soda or potash lye added. Care should be taken that the quantity of copper is not too great, otherwise the peculiar red coloration will be obscured by the blue color of the copper solution.

450. Pancreas Peptone.—The filtrate obtained as described above, by treating the portion of the material insoluble in alcohol with warm water, contains in addition to the albumose and gelatin the whole of the pancreas peptone which may be present. To separate this peptone, 100 cubic centimeters of the aqueous solution are evaporated in a porcelain dish until the volume does not exceed 10 cubic centimeters. When cool, at least 100 cubic centimeters of a saturated cooled solution of ammonium sulfate solution are added, the mixture thoroughly stirred, the precipitate collected upon a filter and washed with a cold saturated solution of ammonium sulfate. The contents of the filter are dissolved in boiling water, the filter thoroughly washed and the filtrate and washings evaporated in a porcelain dish with the addition of barium carbonate until, on the addition of new quantities of barium carbonate, no further trace of ammonia can be discovered. The residue is extracted with water, the barium sulfate and carbonate present separated by filtration, well washed and the nitrogen determined in the evaporated filtrate and washings in the usual way and multiplied by 6.25 to determine the quantity of pancreas peptone.

451. Albumose Peptone.—A part of the albumose peptone which may be present is determined in conjunction with the other bodies mentioned above. The chief quantity is found in the solution of the residue insoluble in alcohol in the following manner:

Fifty cubic centimeters of the solution of this residue in hot water are mixed with an equal volume of dilute sulfuric acid, one volume of acid to three of water, in the cold, and a solution of sodium phosphotungstate added until it produces no further precipitate. The precipitate is washed with dilute sulfuric acid and the nitrogen determined therein. The nitrogen thus found is derived from the albumose, pancreas peptone and gelatin. The quantity of nitrogen in the pancreas peptone and gelatin, as above

described, is subtracted from the total quantity found in the phosphotungstic acid precipitated, and the remainder represents the nitrogen due to the albumose.

452. Nitrogen in the Form of Flesh Bases Insoluble in Alcohol. —

This is determined by subtracting the quantity of nitrogen, determined by the phosphotungstic acid method already described, from the total quantity of nitrogen found in the precipitate insoluble in alcohol and soluble in water.

PART SIXTH

DAIRY PRODUCTS.

453. Introductory.—The importance of dairy products has led to the publication of a vast amount of literature relating thereto, and it seems almost a hopeless task to present even a typical abstract of the various analytical processes which have been proposed and used in their study. The general principles which have been developed in the preceding parts of this volume are applicable to the study of dairy products, and the analyst who is guided by them can intelligently examine the bodies specially considered in the present part. There have been developed, however, many valuable processes for the special examination of dairy products, which are of such a nature that they could not be properly discussed in the preceding pages. In the present part an effort will be made to present in a typical form the most important of these processes and to state the general principles on which they are based. This subject is naturally subdivided into three parts, *viz.*, milk, butter and cheese. The milk sugar industry is not of sufficient importance to receive a special classification.

MILK.

454. Composition of Milk.—The composition of milk not only varies with the genus and species of the mammal from which it is derived, but also depends in a marked degree on idiosyncrasy and on the character of the food and treatment of the animal.²³

Milk is a mixture containing water, proteins, fat, carbohydrates, organic and inorganic acids and mineral salts. There have also been observed in milk in minute quantities ammonia, urea, hypoxanthin, chyme, chyle, biliverdin, cholesterin, mucin, lecithin, kreatin, leucin and tyrosin. In the fermentation which milk undergoes in incipient decomposition there is sometimes devel-

²³ Willey, *Proceedings of the Society for the Promotion of Agricultural Science*, 1889, p. 84.

oped from the protein matter, as pointed out by Vaughn, a ptomaine, tyrotoxon, which is a virulent poison. The nature of this body is not well understood and difficulty has been experienced by other workers in its isolation.²⁴ The presence of these last named bodies is of interest chiefly to the physiologist and pathologist and can receive no further attention here.

From a nutritive point of view, the important components of milk are the fats, proteins and sugar, but especially in the nourishment of the young the value of lime and phosphoric acid must be remembered. The mean composition of the most important milks, as determined by recent analyses, is given below :

	Water. Per cent.	Sugar. Per cent.	Proteins. Per cent.	Fat. Per cent.	Ash. Per cent.
Cow	86.90	4.80	3.60	4.00	0.70
Human	88.75	6.00	1.50	3.45	0.30
Goat.....	85.70	4.45	4.30	4.75	0.80
Ass.....	89.50	6.25	2.00	1.75	0.50
Mare	90.75	5.70	2.00	1.20	0.35
Sheep	80.80	4.90	6.55	6.85	0.90

The mean composition of milk, as given by Watts and König, is given in the following tables :

WATTS.

	Water.	Solids.	Proteins.	Fats.	Sugar.	Mineral Salts.
Woman	87.65	12.35	3.07	3.91	5.01	0.17
Ass	90.70	9.30	1.70	1.55	5.80	0.50
Cow	86.56	13.44	4.08	4.03	4.60	0.73
Goat.....	86.76	13.24	4.33	4.48	3.91	0.62
Sheep.....	83.31	16.69	5.73	6.05	3.96	0.68
Mare	82.84	17.16	1.64	6.87	8.65	

KÖNIG.

	Water.	Fat.	Casein and albumin.	Milk Sugar.	Ash.
Woman	87.41	3.78	2.29	6.21	0.31
Mare	90.78	1.21	1.99	5.67	0.35
Ass	89.64	1.63	2.22	5.99	0.51
Cow	87.17	3.69	3.55	4.88	0.71

The average composition of 120,540 samples of cow milk, as determined by analysis, extending over a period of 11 years, was found by Vieth to be as follows:²⁵

²⁴ Pharmaceutical Journal and Transactions, 1887, Series 3, 18 : 479.

²⁵ The Analyst, 1892, 17 : 85.

	Per cent.
Total solids.....	12.9
Solids not fat	8.8
Fat.....	4.1

The average composition of milk reported by English and other European analysts shows a somewhat lower content of fat than has been found in the United States which is not far from 3.9 per cent. On the other hand, the content of protein in various milk as given by Watts and König is considerably higher than has been reported by other authors.

The quantity of solids and fat in milk is less after longer than after shorter periods between milkings.

The quantity of solids and fat in cow milk is less in the spring than in the autumn.

The chief organic acid naturally present in milk is citric, which exists probably in combination with lime.

The mean content of citric acid in milk is about one-tenth of one per cent.²⁶

Citric acid is not found in human milk, and probably exists only in the mammary secretions of herbivores.

Among the mineral acids of milk, phosphoric is the most important, but a part of the phosphorus found as phosphoric acid in the ash of milk may come from pre-existing organic phosphorus (lecithin, nuclein).

The sulfuric acid, which is found in the ash of milk, is derived from the sulfur of the protein matter during ignition.

Lactic acid is developed from lactose during the souring of milk as the result of bacterial and enzymic activity.

Gases are also found in solution in milk, notably carbon dioxide, which gives to freshly drawn milk its frothy appearance.

The ash of milk has the following composition expressed as grams per liter of the original milk:²⁷

²⁶ Hankel, *Wiener Landwirtschaftliche Zeitung*, 1888 : 401.

Division of Chemistry, Bulletin 24, 1890 : 155.

²⁷ *Die Landwirtschaftlichen Versuchs-Stationen*, 1888, 35 : 352.

Division of Chemistry, Bulletin 24, 1890 : 151.

Component	Grams per liter.	Probable form of combination.	Grams per liter.
Chlorin.....	0.90	{ sodium chlorid potassium chlorid	0.962 0.830
Phosphoric acid....	2.42	{ KH_2PO_4 K_2HPO_4 MgHPO_4 CaHPO_4 $\text{Ca}_3(\text{PO}_4)_2$	1.156 0.853 0.336 0.671 0.806
Potassium	1.80	(as shown above)	
		and as potassium citrate	0.495
Sodium.....	0.49	sodium chlorid	0.962
Lime	1.90	(as shown above)	
		and as calcium citrate	2.133
Magnesia.....	0.20	MgHPO_4	0.336

The percentage composition of the ash of milk, according to Fleischmann and Schrott, is expressed as follows:²⁸

	Per cent.
Potassium oxid, K_2O	25.42
Sodium oxid, Na_2O	10.94
Calcium oxid, CaO	21.45
Magnesium oxid, MgO	2.51
Iron oxid, Fe_2O_3	0.11
Sulfuric acid, SO_3	4.11
Phosphoric acid, P_2O_5	24.11
Chlorin, Cl	14.60
	<hr/> 103.28
Less Cl as O.....	3.28
	<hr/> 100.00

455. Alterability of Milk.—The natural souring and coagulation of milk is attributed by most authorities to bacterial action produced by infection from the air or containing vessels.²⁹ Pasteur shows that fresh milk sterilized at a temperature of 110° may be exposed to the air for some time without danger of souring.³⁰ After about three days, however, a fermentation is set up which is totally different from that produced by the microzymes naturally present in the milk. This point has been further inves-

²⁸ Baumeister, *Milch und Molkerei-Producte*, 1895 : 16.

²⁹ Office of Experiment Stations, *Bulletins* 9 and 25, 1892 and 1895.
Farmers' *Bulletins*, 9 and 29, 1892 and 1895.

³⁰ *Annales de Chimie et de Physique*, 3^e Série, 1862, 64 : 61.

tigated by Béchamp, who finds that the natural souring of milk is accomplished without the evolution of any gas, while the fermentation produced in sterilized milk by the microbes of the air is uniformly attended by a gaseous development.³¹ As a result of his investigations, he concludes that the souring of milk takes place spontaneously by reason of milk being an organic matter, in the physiological sense of the term, and that this alteration is produced solely by the natural microzymes of the milk.

According to Béchamp, the milk derived from healthy animals is capable of spontaneous alteration, which consists in the development of lactic acid and alcohol, and of curd in those milks which contain caseinates produced by the precipitating action of the acids formed. Oxygen and the germs which are present in the air, according to him, have nothing to do with this alteration in the properties of milk. Milk belongs to that class of organic bodies like blood, which are called organic from a physiological point of view, on account of containing automatic forces which produce rapid changes therein when they are withdrawn from the living organisms.

After milk has become sour by the spontaneous action of the microzymes which it contains, there are developed micro-organisms, such as vibriones and bacteria as a natural evolution from the microzymes.

Milk which is sterilized at a high temperature, *viz.*, that of boiling water or above, is no longer milk in the true physiological sense of that term. The globules of the milk undergo changes and the microzymes a modification of their functions, so that in milk thus altered by heat, they are able to produce a coagulation without development of acidity. The microzymes thus modified, however, retain to a large extent their ability to become active. Human milk differs from cow milk in containing neither caseinates nor casein, but special protein bodies, and also a galactozyme or galactozymase functionally very different from that which exists in cow milk. The extractive matter is also a special kind, consisting of milk globules and microzymes belonging particularly to it and containing three times less phosphate and mineral salts

³¹ Bulletin de la Société Chimique de Paris, 3^e Série, 1896, 15 : 248.

than cow milk. Boiling the milk of the cow or other animals does not render it similar to that of woman. There is no treatment of any milk which renders it entirely suited to the nourishment of infants. Béchamp further states that the components of the milk of the cow may be represented by three groups:

1. Organic elements in suspension; consisting chiefly of the globules of the milk, which are mostly composed of the fat, of an epidermoid membrane containing mineral matter of special soluble albumins and of microzymes containing also mineral matter.

2. Dissolved constituents; consisting of caseinates, lactalbuminates, galactozymase holding phosphates in combination, lactose, extractive matter, organic phosphates of lime, acetates, urea and alcohol.

3. Mineral matters in solution; consisting of sodium and calcium chlorids, carbon dioxide and oxygen.³²

It will be noticed from the above classification that Béchamp fails to mention citrate of lime. It is scarcely necessary to add to this brief résumé of the theories of Béchamp that they are entirely at variance with the opinions held by many of the most competent modern authorities.

456. Effects of Boiling on Milk.—On boiling, the albumin in milk is coagulated and on separating the protein bodies by saturation with magnesium sulfate no albumin is found in the filtrate. The total casein precipitated from boiled is therefore greater than from unboiled milk. Jäger has shown that the casein can be precipitated from boiled milk by rennet, even with greater facility than from unboiled.³³ According to this author in 3.75 per cent. of protein in milk there are found 3.15 per cent. of casein, 0.35 of albumin and 0.25 of globulin. Boiling also alters the physiological relations of milk to digestion especially in the case of infants.

457. Appearance of the Milk.—The color, taste, odor and other sensible characters of the milk are to be observed and noted at the time the sample is secured. Any variation from the faint yellow color of the milk is due to some abnormal state. A red-

³² Bulletin de la Société Chimique de Paris, 3^e Série, **15** : 453.

³³ Central-Blatt für medicinische Wissenschaft, 1896, **34** : 146.

dish tint indicates the admixture of blood, while a blue color is characteristic of the presence of unusual micro-organisms. Odor and taste will reveal often the character of the food which the animals have eaten. Any marked departure of the sample from the properties of normal milk should at once lead to its condemnation for culinary or dietetic purposes.

458. Micro-Organisms of the Milk.—Milk is a natural culture solution for the growth of micro-organisms, and they multiply therein with almost incredible rapidity. Some of these are useful, as, for instance, those which are active in the ripening of cream, and others are of an injurious nature, producing fermentations which destroy the sugars or proteins of the milk and develop acid, alcohol, or ptomaine products. It is not possible here to even enumerate the kinds of micro-organisms which abound in milk and the reader is referred to the standard works on that subject.³⁴ Naturally soured milk and pure old fashioned butter-milk have lately been heralded as ideal food and prophylactics of senescence. While such products are palatable to many and undoubtedly wholesome they probably do not have the special values in this direction which have been attributed to them.

For analytical purposes it is important that the sample be kept as free as possible of all micro-organisms, good or bad, which may be accomplished by some of the methods given below.

459. Bacterial Infection.—Modern sanitarians and those concerned with public health problems demand that milk shall be produced and handled under the most rigid sanitary conditions. Without such care this valuable food product may become an active factor in spreading disease producing organisms.

Normal milk as secreted by healthy animals contains relatively few bacteria. Sterile milk has been obtained, by observing surgical precautions, from the udder of living and slaughtered animals. Fore-milk usually contains more bacteria than does middle milk. Certified dairies take advantage of this fact and discard the first streams of milk from each teat in order to reduce the total bacterial content. Bacteria gain entrance to milk on the farm from various sources:—The filth clinging to the

³⁴ Russell, *Outlines of Dairy Bacteriology*, 1902, 5th Edition.

cow's udder and flank, unclean hands of milkers, improperly cleansed buckets and utensils, standing in open containers exposed to stable odors, flies and dust. This evil can be remedied to a large extent by washing and keeping the cow's udder and flank clean, a quiet atmosphere, by insisting on clean hands, by using milk buckets with small openings, and by keeping the milk covered in properly cleansed vessels.

Milk must be cooled at once after milking and subsequently kept in this condition to prevent further bacterial development of the few organisms which gain entrance to the product under the most careful handling. Enormous numbers of bacteria may be found in milk as furnished to large cities. This is mainly due to age, insufficient cooling and improper handling. Such milk when fed to infants and invalids may produce serious intestinal derangements. Numerous articles have been written on the dangers arising from the use of impure milk.^{34a}

460. Bacteriological Examination of Milk.—A committee appointed by the American Public Health Association in 1905 have recommended to the Association a uniform method of bacteriological examination.^{34b}

461. Collection of samples.—The minimum quantity recommended is 10 cubic centimeters, collected in sterile large size test-tubes, well iced until plated. For certified milk, and when possible with market milk, original bottles are preferable. Samples may be removed from cans by using sterile glass tubes 18 inches long properly protected in holders.

462. Media Used.—Plain agar, lactose, or dextrose litmus agar, and lactose litmus gelatin for plating should be prepared according to the standard methods advised. Dextrose or lactose bouillon, or sterilized ox-bile containing one per cent. peptone and two per cent. lactose may be used in fermentation tubes for the determination of the presence of gas producing organisms and streptococci.

^{34a} Milk and Its Relation to Public Health, Hygienic Laboratories, U. S. Public Health Service, Bulletin 56, 1909.

^{34b} American Journal of Public Hygiene, 1910, 20 : 315-345.

463. Plating.—For certified milk dilutions of 1-10, 1-100, and 1-1000 may be used, while for ordinary milk higher dilutions are generally required. It is advisable to use three or more dilutions, ranging above and below the probable count. Dilutions are made by adding one cubic centimeter of milk to nine cubic centimeters sterile water in a test-tube and shaking 25 times, making the first dilution of 1-10; from this as many dilutions may be made as thought necessary. Porous earthenware dishes absorb excess moisture and may be used if desired. A sufficient number of graduated pipettes containing one cubic centimeter and five cubic centimeter quantities should be prepared for use.

Incubation.—Agar plates and liquid cultures may be either incubated at 37° for 48 hours or at 21° for five days. Gelatin plates should be kept at about 20° and observed daily to note the presence of liquifiers.

Counting colonies.—Various counting devices are placed on the market. Use some simple method. Select a plate containing as near 50 colonies as possible for counting purposes and express results by thousands, ten-thousands, etc.

Direct Examination.—Centrifugal specimens may be stained and examined from definite quantities of milk if desired to obtain an approximate number of the bacteria present before plating or where plating is impracticable.

Fermentation tests.—The detection of the presence of fecal organisms may be made by inoculating one cubic centimeter quantity of appropriate solutions into either two per cent. sugar bouillon or ox-bile in fermentation tubes. Small inverted test-tubes within a larger tube are the most convenient form of fermentation tube. Gas should be noted after 48 hours incubation and stained smears made from each dilution and examined for the presence of streptococci.

464. Bacterial Count.—Certified milk should contain less than 10,000 bacteria per cubic centimeter. Ordinary market milk may contain millions of organisms. Sanitation aims at reducing the germ content to the minimum, thus lessening the possibilities of disease transmission through this medium. In established

bacterial standards for commercial milk, above 500,000 bacteria per cubic centimeter is considered the maximum number permissible, while certain others have adopted standards ranging from 100,000 to 500,000 bacteria per cubic centimeter. Milk containing bacteria less than the number indicated may yet harbor pathogenic varieties, such as tuberculosis and typhoid bacilli which would render it a more dangerous article for consumption than a milk containing a high count of such organisms as the lactic acid group. So it is not alone the number of bacteria present, but the kind which is the most important factor in determining a sanitary milk. The presence of colon bacilli shows the presence of fecal contamination, while milk containing excessive streptococci generally indicates inflammatory conditions of the udder. Milk containing more than 500,000 bacteria per cubic centimeter with an excessive number of colon types and streptococci should be condemned as being a filthy, decomposed and putrid substance.

465. Sampling Milk.—It is not difficult to secure for examination representative samples of milk, if the proper precautions be taken. On the other hand, the ease and rapidity with which a milk undergoes profound changes render necessary a careful control of the methods of sampling. The most rapid changes to which a mass of milk is obnoxious are due to the separation of the fat particles and to the action of bacteria. Even after standing for a few minutes, it will be found that the fat globules are not evenly distributed. Before securing the sample for analysis, it is necessary to well stir or mix the milk. A mean sample may also be secured from a can of milk by the sampling tube devised by Scovell, described below.

466. Scovell's Milk Sampler.—In sampling large quantities of milk in pails or shipping cans, it is exceedingly inconvenient to mix the milk by pouring from one vessel to another or by any easy process of stirring. In order to get representative samples in such conditions, Scovell has put in use a sampler, by means of which a typical portion of the milk may be withdrawn from a can without either pouring or stirring. The construction of the sampler is shown in Fig. 99, representing it in outline and longi-

tudinal section. The tube *a*, made of brass, is open at both ends and of any convenient dimensions. Its lower end slides in a large tube *b*, closed at the bottom and having three elliptical, lateral openings *c*, which admit the milk as the tube is slowly depressed in the contents of the can. In getting the sample, *a* is raised and when the bottom of *b* reaches the bottom of the can *a* is pushed down as shown in the section. The milk contained in the sampler is then readily withdrawn.

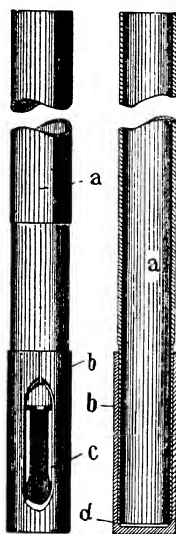


Fig. 99.—Scovell's Milk Sampling Tube

In securing samples, a full detailed description of the cow or herd furnishing them is desirable, together with all other data which seem to illustrate in any way the general and particular conditions of the dairy. Samples are to be preserved in clean, well stoppered vessels, properly numbered and securely sealed.

467. Preserving Milk for Analysis.—Pasteurizing or boiling the sample is not advisable by reason of the changes produced in the milk by heat. The milk sample may be preserved by adding to it a little chloroform, one part in 100 being sufficient. Boric and salicylic acids may also be used, but not so advantageously as

formaldehyd or mercuric chlorid. Rideal has observed that one part of formaldehyd will preserve 10,000 parts of milk in a fresh state for seven days. The formaldehyd sold in the trade contains about one part of formaldehyd in 320 of the mixture. One-half pint of this commercial article is sufficient for about 20 gallons of milk, corresponding to about one part of pure formaldehyd to 45,000 parts of milk. Rideal much prefers formalin (formaldehyd) to borax or boric acid as a milk preservative. No preservative of any kind should be admitted in milk intended for food purposes.

Samples of milk can be kept in this way from four to six weeks by adding about one drop of the commercial formaldehyd to each ounce of sample. The analyst should remember in such cases that the formaldehyd may not all escape on evaporation, on account of forming some kind of a compound with the constituents of the milk, as is pointed out by Bevan.³⁵

Bevan suggests that the formaldehyd may not actually be retained in the sample, but that the increase in the apparent amount of total solids is due to the conversion of the lactose into galactose. This point, however, has not been determined. One gram of fine-ground mercuric chlorid dissolved in 2,000 grams of milk will preserve milk, practically unchanged, for several days. One gram of potassium bichromate dissolved in one liter of milk will also preserve it for some time. Thymol, hydrochloric acid, carbon disulfid, ether and other antiseptics may also be employed. No more of the preserving agent should be used than is required to keep the milk until the analysis is completed.

All methods of preservation are rendered more efficient by the maintenance of a low temperature, whereby the vitality of the bacteria is greatly reduced.

468. Detection of Formaldehyd.—Richmond and Boseley propose to detect formalin by means of diphenylamin. A solution of diphenylamin is made with water, with the help of just enough sulfuric acid to secure a proper solvent effect. The liquid to be tested, which is supposed to contain formaldehyd, or the distillate therefrom, is added to this solution and boiled. If formal-

³⁵ The Analyst, 1895, 20 : 152.

dehyd be present, a white flocculent precipitate is deposited, which is colored green if the acid used contain nitrates.

The official method of detecting formaldehyde is as follows:³⁶

(a) *Preparation of Sample*.—If the material be solid or semi-solid, tritrate from 200 to 300 grams in a mortar with about 100 cubic centimeters of water until a sufficient degree of fluidity is obtained. Transfer to a short-necked distilling flask of copper or glass of from 500 to 800 cubic centimeters capacity and make distinctly acid with phosphoric acid. Connect the flask with a glass condenser and distil from 40 to 50 cubic centimeters.

(b) *Phenylhydrazin Hydrochlorid Method*.³⁷—Mix five cubic centimeters of the distillate as prepared under (a), or of an alcoholic solution or extract from the substance under examination, with 0.03 gram of phenylhydrazin hydrochlorid, and four or five drops of a one per cent. solution of ferric chlorid. Add slowly and with agitation, in a bath of cold water to prevent the heating of the liquid, from one to two cubic centimeters of concentrated sulfuric acid. A precipitate is formed which can be dissolved by the addition of either concentrated sulfuric acid (keeping the mixture cool) or with alcohol. With meats and fats the formaldehyd should first be extracted with alcohol and the filtrate tested. In the case of fat it is necessary to heat the mixture above the melting point of the fat to insure thorough extraction. Milk is shaken with an equal volume of strong alcohol and the filtrate employed. Other liquids are shaken with an equal volume of strong alcohol and filtered in case of the formation of any insoluble matter.

In the hands of different analysts this method is found to give reliable reactions for formaldehyd in solutions of formaldehyd varying from one part in 50,000 to one part in 150,000. Acetic aldehyd and benzaldehyd give no reaction when treated by this method and do not interfere with the reaction given by formaldehyd.

³⁶ Bureau of Chemistry, Bulletin 107, 1912 : 183.

³⁷ Arnold and Mentzel, *Zeitschrift für Nahrung und Genussmittel*, 1902, 5 : 353.

(c) *Phenylhydrazin Hydrochlorid and Ferricyanid Method*.³⁸

—This method may be applied directly to liquid foods or to an aqueous or alcoholic extract of solid foods. To from three to five cubic centimeters of liquid food or extract of the same add a lump of phenylhydrazin hydrochlorid about the size of a pea, from two to four drops (not more) of a five to 10 per cent. solution of potassium ferricyanid, and from eight to 12 drops of an approximately 12 per cent. solution of sodium hydroxid. The method is not applicable to preparations containing blood-coloring matter. In such cases use nitroprussid in place of the ferricyanid. Alcoholic extracts from foods must be diluted with water to prevent the precipitation of potassium ferricyanid.

Apply the method directly to milk without any preparation of sample. In the case of meat finely comminute the sample, extract with two volumes of hot water, and employ the liquid pressed out for the test. Warm fats above the melting point with 10 cubic centimeters of alcohol (80 to 95 per cent. by volume), thoroughly shake, cool, and filter through a moistened paper, using the filtrate for the examination.

When formaldehyd is present to the extent of more than one part in 70,000 to 80,000 in the solution tested, a distinct green or bluish green reaction is obtained. In more dilute solutions the green tint becomes less marked and a yellow tinge tending toward greenish brown is formed.

With this method acetic aldehyd and benzaldehyd give a color varying from red to brown, according to the strength of the solution. A reaction may therefore be obtained with these aldehyds similar to that obtained with formaldehyd in solutions more dilute than one part in 70,000. The presence of acetic aldehyd or benzaldehyd together with formaldehyd gives a yellowish or yellowish green tinge. The reaction for formaldehyd may therefore be masked by the presence of other aldehyds, but is characteristic when a clear green color is obtained.

(d) *Hegner's Method*.³⁹—To the milk to be tested add strong

³⁸ Arnold and Mentzel, *Chemiker-Zeitung*, 1902, **26** : 246; Abstracts Journal Chemical Society, 1902, **82** [2] : 367; Abstracts *Chemisches Centralblatt*, 1902, **73** [1] : 1076.

³⁹ Analyst, 1895, **20** 155.

commercial sulfuric acid without mixing, and at the junction of the two liquids a violet or blue color will appear if the milk contains one or more parts of formaldehyd per 10,000. This color is supposed to be given only when there is a trace of ferric chlorid or other oxidizing agent present. As pointed out by Hehner, milk may be treated directly by this method without any other operation, and some other articles of food rich in proteins—for example, egg albumen—give the reaction in the presence of water without the addition of milk. The distillate described above may be mixed with milk and this test applied.

(e) *Leach's Method*.—Add about five cubic centimeters of the distillate obtained under (a) to an equal volume of pure milk in a porcelain casserole and about 10 cubic centimeters of concentrated hydrochloric acid, containing one cubic centimeter of 10 per cent. ferric chlorid solution, to each 500 cubic centimeters of acid. Heat to 80° or 90° directly over the gas flame, giving the casserole a rotary motion to break up the curd. A violet coloration indicates formaldehyd.

(f) *Rimini's Method*.⁴⁰—Treat 15 cubic centimeters of milk or other liquid food under examination or of the distillate prepared as directed under (a) with one cubic centimeter of a dilute solution of phenylhydrazin hydrochlorid, then with a few drops of dilute ferric-chlorid solution, and, finally, with concentrated hydrochloric acid. The presence of formaldehyd is indicated by the formation of a red color, which changes after some time to orange yellow.

This method is suitable for the examination of milk without previous treatment, but more delicate tests may be obtained from the distillate from milk or from milk serum. The reaction is not interfered with by acetic aldehyd or benzaldehyd.

(g) *Phloroglucol Method*.⁴¹—Prepare the reagent by dissolving one gram phloroglucol and 20 grams of sodium hydroxid in sufficient water to make 100 cubic centimeters. To 10 cubic

⁴⁰ Ann. di Farmacol., 1898, 97 : Abstracts Chemisches Centrblatt, 1898, 69 [1] : 1152 ; Abstracts Journal Society Chemical Industry, 1898, 17 : 697.

⁴¹ Jorissen, Service de Surveillance des Aliments en Belgique, through Bulletin de la Societe Chimique des Belges, 1897-98, 11 : 12, 211 ; Abstracts Analyst, 1897, 22 : 282.

centimeters of milk or other liquid food under examination in a test-tube add, by means of a pipette, two cubic centimeters of this reagent, placing the end of the pipette on the bottom of the tube in such a manner that the reagent will form a separate layer.

A bright red coloration (not purple) is formed at the zone of contact if formaldehyd be present. This solution gives a yellow color in the presence of some other aldehyds, and if it is used for the detection of aldehyd formed by the oxidation of methyl alcohol after the destruction of ethyl aldehyd with hydrogen peroxid an orange-yellow color will slowly appear when an insufficient amount of hydrogen peroxid has been employed. On the other hand, if the excess of hydrogen peroxid is not fully destroyed before the use of this reagent a purple color will slowly form. The clear, red color given by the use of this reagent forms quickly, and in the presence of but a small amount of formaldehyd soon fades.

469. Freezing Point of Milk.—By reason of its content of sugar and other dissolved solids, the freezing point of milk is depressed below 0° . A good idea of the purity of whole milk is secured by subjecting it to a kryoscopic test. The apparatus employed for this purpose is that used in general analytical work in the determination of freezing points. Pure full milk freezes at about 0.55 below zero, and any marked variation from this number shows adulteration or abnormal composition.⁴² A simple apparatus, especially adapted to milk, is described by Beckmann.⁴³ The kryoscopic investigation may also be extended to butter fat dissolved in benzol.

470. Electric Conductivity of Milk.—The electric conductivity of milk may also be used as an index of its composition. The addition of water to milk diminishes its conductivity.⁴⁴ This method of investigation has at present but little practical value.

471. Viscosity of Milk.—The viscosity of milk may be determined by the methods already described. Any variation from

⁴² Forschungs-Berichte über Lebensmittel etc., 1895, 2 : 368.

⁴³ Forschungs-Berichte über Lebensmittel etc., 1894, 1 : 422.

⁴⁴ Forschungs-Berichte über Lebensmittel etc., 1895, 2 : 372.

the usual degree of fluidity is indicated either by the abstraction of some of the contents of the milk, the addition of some adulterant or the result of fermentation.

472. Acidity and Alkalinity of Milk.—Fresh milk of normal constitution has an amphoteric reaction. It will redden blue and blue red litmus paper. This arises from the presence in the milk of both neutral and acid phosphates of the alkalies. A saturated alkaline phosphate, *i. e.*, one in which all the acid hydrogen of the acid has been replaced by the base, has an alkaline reaction while the acid phosphates react acid. When fresh milk is boiled its reaction becomes strongly alkaline and this arises chiefly from the escape of the dissolved carbon dioxid. By the action of micro-organisms on the lactose of milk, the alkaline reaction soon becomes acid, and delicate test paper will show this decomposition long before it becomes perceptible to the taste. It is advisable to test the reactions of the milk as soon as possible after it is drawn from the udder, both before and after boiling.

473. Determination of the Acidity of Milk.—In the determination of the acidity of milk it is important that it first be freed of the carbon dioxid it contains.⁴⁵ Van Slyke has found that too high results are obtained by the direct titration of milk for acidity, and when the milk is previously diluted the results are also somewhat too high.⁴⁶ Good results are got by diluting the milk with hot water and boiling for a short time to expel the carbon dioxid. Twenty-five cubic centimeters of milk are diluted with water to about a quarter of a liter, as above, two cubic centimeters of a one per cent. alcoholic phenolphthalein added and the titration accomplished by decinormal alkali. This variation of the methods of procedure, suggested by Hopkins and Powers, appears to be the best process at present known for the determination of acidity. The reader is referred to the paper cited above for references to other methods which have been proposed.

474. Opacity of Milk.—The white color and opacity of milk are doubtless due to the presence of the suspended fat particles and to the colloid casein. On the latter it is probably principally

⁴⁵ Hopkins and Powers, Division of Chemistry, Bulletin 47, 1896 : 127.

⁴⁶ Division of Chemistry, Bulletin 38, 1893 : 118.

dependent since the color of milk is not very sensibly changed after it has passed the extractor, which leaves not to exceed one-tenth of one per cent. of fat in it. Some idea of the quality of the milk, however, may be obtained by determining its opacity. This is accomplished by the use of a lactoscope. The one generally employed was devised by Feser and is shown in Fig. 100.

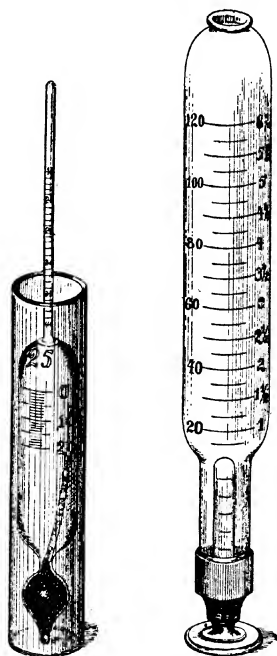


Fig. 100.—Lactoscope, Lactometer and Creamometer.

The instrument consists of a cylindrical glass vessel of a little more than 100 cubic centimeters content, in the lower part of which is set a cone of white glass marked with black lines. Into this part are placed four cubic centimeters of milk. A small quantity of water is added and the contents of the vessel shaken. This operation is repeated until the black lines on the white glass just become visible. The graduations on the left side show the volume of water which is necessary to bring the dark lines into

view, while those on the right indicate approximately the percentage of fat present.

Among the other lactoscopes which have been used may be mentioned those of Donné, Vogel, Hoppe-Seyler, Trommer, Seidlitz, Reischauer, Mittelstrass, Hénocque, and Heusner.⁴⁷ Since the invention of so many quick and accurate methods of fat estimation these instruments have little more than a historical interest.

475. Creamometry.—The volume of cream which a sample of milk affords under arbitrary conditions of time and temperature is sometimes of value in judging the quality of milk. A convenient creamometer is a small cylinder graduated in such a way that the volume of cream separated in a given time can be easily noted. There are many kinds of apparatus used for this purpose, a typical one being shown in Fig. 100 on the left.

The usual time of setting is 24 hours. A quicker determination is secured by placing the milk in strong glass graduated tubes and subjecting these to centrifugal action. The process is not exact and is now rarely practiced as an analytical method, even for valuing the butter making properties of milk.

476. Specific Gravity.—The specific gravity of milk is usually referred to a temperature of 15° (60° F.). It would be better to substitute 20° for 15°, since the former temperature more nearly represents the average temperature of the laboratory. Generally no attempt is made to free the milk of dissolved gases beforehand. This should not be done by boiling but by placing the sample in a vacuum for some time. Any of the methods described for determining specific gravity in sugar solutions may be used for milk (63-75). The specific gravity of milk varies in general from 1.028 to 1.034. Nearly all good cow milk from herds will show a specific gravity varying from 1.030 to 1.032. In extreme cases from single cows the limits may exceed those first given above, but such milk cannot be regarded as normal.

Increasing quantities of solids not fat in solution tend to increase the specific gravity, while an excess of fat tends to diminish

⁴⁷ Becke, *Die Milchprüfung-Methoden*, 1882 : 45.

Rouvier, *Le Lait*, 1893 : 45.

it. There is a general ratio existing between the solids not fat and the fat in cow milk, which may be expressed as 9:4. The removal of cream and the addition of water in such a manner as not to affect the specific gravity of the sample disturbs this ratio.

The determination of the specific gravity alone, therefore, cannot be relied upon as an index of the purity of a milk.

477. Lactometry.—A hydrometer especially constructed for use in determining the density of milk is called a lactometer. In this country the one most commonly used is known as the lactometer of the New York Board of Health. It is a hydrometer, delicately constructed, with a large cylindrical air space and a small stem carrying the thermometric and lactometric scales. The milk is brought to a temperature of 60° F. and the reading of the lactometer scale observed. This is converted into a number expressing the specific gravity by means of a table of corresponding values given below. Each mark on the scale of the instrument corresponds to two degrees and these marks extend from 0° to 120°. The numbers of this scale can be converted into those corresponding to the direct reading instrument, described in the next paragraph, by multiplying them by 0.29.

TABLE SHOWING SPECIFIC GRAVITIES CORRESPONDING TO DEGREES OF THE NEW YORK BOARD OF HEALTH LACTOMETER. TEMPERATURE 60° F.

Degree.	Sp. gr.	Degree.	Sp. gr.
90	1.02619	106	1.03074
91	1.02639	107	1.03103
92	1.02668	108	1.03132
93	1.02697	109	1.03161
94	1.02726	110	1.03190
95	1.02755	111	1.03219
96	1.02784	112	1.03248
97	1.02813	113	1.03277
98	1.02842	114	1.03306
99	1.02871	115	1.03335
100	1.02900	116	1.03364
101	1.02929	117	1.03393
102	1.02958	118	1.03422
103	1.02987	119	1.03451
104	1.03016	120	1.03480
105	1.03045		

The minimum density for whole milk at 60° F. is fixed by this instrument at 100°, corresponding to a specific gravity of 1.029. The instrument is also constructed without the thermometric scale. The mean density of many thousand samples of pure milk, as observed by the New York authorities, is 1.0319.

The specific gravity is easily secured, and while not of itself decisive, should always be determined. The specific gravity of milk increases for some time after it is drawn and should be made both when fresh and after the lapse of several hours.⁴⁸

478. Direct Reading Lactometer.—A more convenient form of lactometer is one which gives the specific gravity directly on the scale. The figures given represent those found in the second and third decimal places of the number expressing the specific gravity. Thus 31 on the scale indicates a specific gravity of 1.031. This instrument is also known as the lactometer of Quévenne. For use with milk, the scale of the instrument does not need to embrace a wider limit than from 25 to 35, and such an instrument is capable of giving more delicate readings than when the scale extends from 14 to 42, as is usually the case with the Quévenne instrument.

Langlet has invented a lactoscope with a scale, showing the corrections to be applied for temperatures other than 15°. A detailed description of this instrument, as well as the one proposed by Pinchon, is found in the original papers.⁴⁹

479. Density of Sour Milk.—Coagulated milk cannot be used directly for the determination of the specific gravity, both because of its consistence and by reason of the fact that the fat is more or less completely separated. In such a case, the casein may be dissolved by the addition of a measured quantity of a solvent of a known specific gravity, the density of the resulting solution determined and that of the original milk calculated from the observed data. Ammonia is a suitable solvent for this purpose.⁵⁰

480. Density of the Milk Serum.—The specific gravity of the milk serum, after the removal of the fat and casein by precipita-

⁴⁸ The Analyst, 1890, 15 : 170.

⁴⁹ Rouvier, Le Lait, 1893 : 35.

⁵⁰ Central-Blatt für Nahrungs- und Genussmittel Chemie, 13 : 277.

tion and filtration, may also be determined. For normal cow milk the number is about 1.027.

481. Total Solids.—The direct gravimetric determination of the total solids in milk is attended with many difficulties, and has been the theme of a very extended periodical literature. A mere examination of the many processes which have been proposed would require several pages.

The most direct method of procedure is to dry a small quantity of milk in a flat-bottom dish to constant weight on a steam-bath. The surface of the dish should be very large, even for one or two grams of milk; in fact the relation between the quantity of milk and the surface of the dish should be such that the fluid is just sufficient in amount to moisten the bottom of the dish with the thinnest possible film. The dish, during drying, is kept in a horizontal position at least until its contents will not flow. The water of the sample will be practically all evaporated in about two hours. The operation may be accelerated by drying in vacuo.

The drying may also be accomplished by using a flat-bottom dish containing some absorbent, such as sand, pumice stone, asbestos or crysolite. The milk may also be absorbed by a dried paper coil and dried thereon.

It is convenient to determine the water in the sample subsequently to be used for the gravimetric determination of the fat, and this is secured by the adoption of the paper coil method, as suggested by the author, or by the use of a perforated metal tube containing porous asbestos, as proposed by Babcock.⁵¹

The process applied also to the determination of the fat is conveniently carried out as follows:

Provide a hollow cylinder of perforated sheet metal 60 millimeters long and 20 millimeters in diameter, closed five millimeters from one end by a disk of the same material. The perforations should be about 0.7 millimeter in diameter and as close together as possible. Fill loosely with from one and a half to two and a half grams of dry woolly asbestos and weigh. Introduce a weighed quantity of milk (about five grams). Dry at 100° for four hours. During the first part of the drying the

⁵¹ Division of Chemistry, Bulletin 46, 1895 : 36.

door of the oven should be left partly open to allow escape of moisture. Cool in a desiccator and weigh. Repeat the drying until the weight remains constant. Place in an extractor and treat with anhydrous ether for two hours. Evaporate the ether and dry the fat at 100°. The extracted fat is weighed and the number thus obtained may be checked by drying and weighing the cylinder containing the residue.

The asbestos best suited for use in this process should be of a woolly nature, quite absorbent, and, previous to use, be ignited to free it of moisture and organic matter. A variety of serpentine, crysolite, is sometimes used instead of asbestos. When the content of water alone is desired, it is accurately determined by drying in vacuo over pumice stone.

The methods above mentioned are typical and will prove a sufficient guide for conducting the desiccation, either as described or by any modification of the methods which may be preferred.

482. Calculation of Total Solids.—By reason of the ease and celerity with which the density of a milk and its content of fat can be obtained, analysts have found it convenient to calculate the percentage of total solids instead of determining it directly. This is accomplished by arbitrary formulas based on the data of numerous analyses. These formulas give satisfactory results when the samples do not vary widely from the normal and may be used with advantage in most cases.

Among the earliest formulas for the calculation may be mentioned those of Fleischmann and Morgen,⁵² Behrend and Morgen,⁵³ Claus, Stutzer and Meyer,⁵⁴ Hehner,⁵⁵ and Hehner and Richmond.⁵⁶ Without doing more than citing these papers it will be sufficient here to give the formulas as corrected by the most recent experience.

In the formula worked out by Babcock the specific gravity of

⁵² *Journal für Landwirtschaft*, 1882, **30** : 293 ; 1885, **33** : 251.

⁵³ *Journal für Landwirtschaft*, 1879, **27** : 249.

⁵⁴ *Forschungen auf dem Gebiete der Viehhaltung*, 1879 : 265.

⁵⁵ *The Analyst*, 1882, **7** : 129.

⁵⁶ *The Analyst*, 1888, **13** : 26.

the sample is represented by S , the fat by F , and the solids not fat by t . The formula is written as follows:⁵⁷

$$t = \left(\frac{100 S - FS}{100 - 1.0753 FS} - 1 \right) (250 - 2.5 F).$$

In this formula it is assumed that the difference between the specific gravity of the milk serum and that of water is directly proportional to the per cent. of solids in the serum, but this assumption is not strictly correct. Even in extreme cases, however, the error does not amount to more than 0.05 per cent.

Since a given amount of milk sugar increases the density of a milk more than the same quantity of casein, it is evident that the formula would not apply to those instances in which the ratio between these two ingredients is greatly disturbed, as for instance, the whey.

The formula of Hehner and Richmond, in its latest form, is expressed as follows: $T = 0.2625 \frac{G}{D} + 1.2 F$, in which T represents the total solids, G the reading of the Quévenne lactometer, D the specific gravity, and F the fat.

Example.—Let the reading of the lactometer be 31, corresponding to D 1.031, and the percentage of fat be three and five-tenths, what is the percentage of the total solids?

Substituting these values in the formulas we have

$$T = 0.2625 \frac{31}{1.031} + (1.2 \times 3.5) = 12.09.$$

To simplify the calculations, Richmond's formula may be written $T = \frac{G}{4} + \frac{6 F}{5} + 0.14$. Calculated by this shortened formula from the above data $T = 12.09$, the same as given in the larger formula.

Calculating the solids not fat in the hypothetical case given above by Babcock's formula, we get $t = 8.46$, and this plus 3.5 gives 11.96, which is slightly lower than the number obtained by the Richmond process.

⁵⁷ Division of Chemistry, Bulletin 47, 1896 : 123.

The Babcock formula may be simplified by substituting the number expressing the reading of the Quévenne lactometer for that indicating the specific gravity, in other words, the specific gravity multiplied by 100 and the product diminished by 1,000.

The formulas for solids not fat and total solids then become

$$t = \frac{L}{4} + 0.2 F, \text{ and } T = \frac{L}{4} + 1.2 F, \text{ in which } L \text{ represents}$$

the reading of the lactometer. By the addition of the constant factor 0.14 the results calculated by the formula of Babcock are the same as those obtained by the method of Richmond.

In the following table are given the solids not fat in milks as calculated by Babcock's formula. To obtain the total solids add the per cent. of fat to solids not fat. To obtain total solids according to Richmond's formula increase the number by 0.14.

TABLE SHOWING PER CENT. OF SOLIDS NOT FAT IN MILK CORRESPONDING TO QUÉVENNE'S LACTOMETER READINGS AND PER CENT. OF FAT.

Per cent. of fat.	Lactometer reading at 60° F.										
	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	36.
0.0	6.50	6.75	7.00	7.25	7.50	7.75	8.00	8.25	8.50	8.75	9.00
0.1	6.52	6.77	7.02	7.27	7.52	7.77	8.02	8.27	8.52	8.77	9.02
0.2	6.54	6.79	7.04	7.29	7.54	7.79	8.04	8.29	8.54	8.79	9.04
0.3	6.56	6.81	7.06	7.31	7.56	7.81	8.06	8.31	8.56	8.81	9.06
0.4	6.58	6.83	7.08	7.33	7.58	7.83	8.08	8.33	8.58	8.83	9.08
0.5	6.60	6.85	7.10	7.35	7.60	7.85	8.10	8.35	8.60	8.85	9.10
0.6	6.62	6.87	7.12	7.37	7.62	7.87	8.12	8.37	8.62	8.87	9.12
0.7	6.64	6.89	7.14	7.39	7.64	7.89	8.14	8.39	8.64	8.89	9.14
0.8	6.66	6.91	7.16	7.41	7.66	7.91	8.16	8.41	8.66	8.91	9.16
0.9	6.68	6.93	7.18	7.43	7.68	7.93	8.18	8.43	8.68	8.93	9.18
1.0	6.70	6.95	7.20	7.45	7.70	7.95	8.20	8.45	8.70	8.95	9.20
1.1	6.72	6.97	7.22	7.47	7.72	7.97	8.22	8.47	8.72	8.97	9.22
1.2	6.74	6.99	7.24	7.49	7.74	7.99	8.24	8.49	8.74	8.99	9.24
1.3	6.76	7.01	7.26	7.51	7.76	8.01	8.26	8.51	8.76	9.01	9.26
1.4	6.78	7.03	7.28	7.53	7.78	8.03	8.28	8.53	8.78	9.03	9.28
1.5	6.80	7.05	7.30	7.55	7.80	8.05	8.30	8.55	8.80	9.05	9.30
1.6	6.82	7.07	7.32	7.57	7.82	8.07	8.32	8.57	8.82	9.07	9.32
1.7	6.84	7.09	7.34	7.59	7.84	8.09	8.34	8.59	8.84	9.09	9.34
1.8	6.86	7.11	7.36	7.61	7.86	8.11	8.36	8.61	8.86	9.11	9.37
1.9	6.88	7.13	7.38	7.63	7.88	8.13	8.38	8.63	8.88	9.14	9.39
2.0	6.90	7.15	7.40	7.65	7.90	8.15	8.40	8.66	8.91	9.16	9.41
2.1	6.92	7.17	7.42	7.67	7.92	8.17	8.42	8.68	8.93	9.18	9.43
2.2	6.94	7.19	7.44	7.69	7.94	8.19	8.44	8.70	8.95	9.20	9.45

Per cent. of fat.	Lactometer reading at 60° F.														
	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	36.				
2.3	6.96	7.21	7.46	7.71	7.96	8.21	8.46	8.72	8.97	9.22	9.47				
2.4	6.98	7.23	7.48	7.73	7.98	8.23	8.48	8.74	8.99	9.24	9.49				
2.5	7.00	7.25	7.50	7.75	8.00	8.25	8.50	8.76	9.01	9.26	9.51				
2.6	7.02	7.27	7.52	7.77	8.02	8.27	8.52	8.78	9.03	9.28	9.53				
2.7	7.04	7.29	7.54	7.79	8.04	8.29	8.54	8.80	9.05	9.30	9.55				
2.8	7.06	7.31	7.56	7.81	8.06	8.31	8.57	8.82	9.07	9.32	9.57				
2.9	7.08	7.33	7.58	7.83	8.08	8.33	8.59	8.84	9.09	9.34	9.59				
3.0	7.10	7.35	7.60	7.85	8.10	8.36	8.61	8.86	9.11	9.36	9.61				
3.1	7.12	7.37	7.62	7.87	8.13	8.38	8.63	8.88	9.13	9.38	9.64				
3.2	7.14	7.39	7.64	7.89	8.15	8.40	8.65	8.90	9.15	9.41	9.66				
3.3	7.16	7.41	7.66	7.92	8.17	8.42	8.67	8.92	9.18	9.43	9.68				
3.4	7.18	7.43	7.69	7.94	8.19	8.44	8.69	8.94	9.20	9.45	9.70				
3.5	7.20	7.45	7.71	7.96	8.21	8.46	8.71	8.96	9.22	9.47	9.72				
3.6	7.22	7.48	7.73	7.98	8.23	8.48	8.73	8.98	9.24	9.49	9.74				
3.7	7.24	7.50	7.75	8.00	8.25	8.50	8.75	9.00	9.26	9.51	9.76				
3.8	7.26	7.52	7.77	8.02	8.27	8.52	8.77	9.02	9.28	9.53	9.78				
3.9	7.28	7.54	7.79	8.04	8.29	8.54	8.79	9.04	9.30	9.55	9.80				
4.0	7.30	7.56	7.81	8.06	8.31	8.56	8.81	9.06	9.32	9.57	9.83				
4.1	7.32	7.58	7.83	8.08	8.33	8.58	8.83	9.08	9.34	9.59	9.85				
4.2	7.34	7.60	7.85	8.10	8.35	8.60	8.85	9.11	9.36	9.62	9.87				
4.3	7.36	7.62	7.87	8.12	8.37	8.62	8.88	9.13	9.38	9.64	9.89				
4.4	7.38	7.64	7.89	8.14	8.39	8.64	8.90	9.15	9.40	9.66	9.91				
4.5	7.40	7.66	7.91	8.16	8.41	8.66	8.92	9.17	9.42	9.68	9.93				
4.6	7.43	7.68	7.93	8.18	8.43	8.68	8.94	9.19	9.44	9.70	9.95				
4.7	7.45	7.70	7.95	8.20	8.45	8.70	8.96	9.21	9.46	9.72	9.97				
4.8	7.47	7.72	7.97	8.22	8.47	8.72	8.98	9.23	9.48	9.74	9.99				
4.9	7.49	7.74	7.99	8.24	8.49	8.74	9.00	9.25	9.50	9.76	10.01				
5.0	7.51	7.76	8.01	8.26	8.51	8.76	9.02	9.27	9.52	9.78	10.03				
5.1	7.53	7.78	8.03	8.28	8.53	8.79	9.04	9.29	9.54	9.80	10.05				
5.2	7.55	7.80	8.05	8.30	8.55	8.81	9.06	9.31	9.56	9.82	10.07				
5.3	7.57	7.82	8.07	8.32	8.57	8.83	9.08	9.33	9.58	9.84	10.09				
5.4	7.59	7.84	8.09	8.34	8.60	8.85	9.10	9.36	9.61	9.86	10.11				
5.5	7.61	7.86	8.11	8.36	8.62	8.87	9.12	9.38	9.63	9.88	10.13				
5.6	7.63	7.88	8.13	8.39	8.64	8.89	9.15	9.40	9.65	9.90	10.15				
5.7	7.65	7.90	8.15	8.41	8.66	8.91	9.17	9.42	9.67	9.92	10.17				
5.8	7.67	7.92	8.17	8.43	8.68	8.94	9.19	9.44	9.69	9.94	10.19				
5.9	7.69	7.94	8.20	8.45	8.70	8.96	9.21	9.46	9.71	9.96	10.22				
6.0	7.71	7.96	8.22	8.47	8.72	8.98	9.23	9.48	9.73	9.98	10.24				

483. Determination of Ash.—In the combustion of the solid residue obtained by drying milk, it is important to observe the directions already given (36-46).

In the direct ignition of the sample, a portion of the sulfur and

phosphorus may escape oxidation and be lost as volatile compounds. This loss may be avoided by the use of proper oxidizing agents or by conducting the combustion as heretofore described.⁵⁸ In the official method, it is directed to add six cubic centimeters of nitric acid to 20 of milk, evaporate to dryness and ignite the residue at a low red heat until free of carbon.⁵⁹ It is doubtful if this precaution be entirely sufficient to save all the sulfur and phosphorus, but the method is evidently more reliable than the common one of direct ignition without any oxidizing reagent whatever.

484. Form of Fat in Milk.—The fat in milk occurs in the form of globules suspended in the liquid, in other words, as an emulsion. Many authorities have asserted that each globule of fat is contained in a haptogenic membrane composed presumably of nitrogenous matter, but there is no convincing evidence of the truth of this opinion. The weight of experimental evidence is in the opposite direction. The supposed action of the membrane and the phenomena produced thereby are more easily explained by the surface tension existing between the fat globules and the menstruum in which they are suspended.

Babcock affirms that the spontaneous coagulation of the fibrin present in milk tends to draw the fat globules into clusters, and this tendency can be arrested by adding a little soda or potash lye to the milk as soon as it is drawn.⁶⁰

The diameter of the fat globules is extremely variable, extending in some cases from two to 20 micromillimeters. In cow milk, the usual diameters are from three to five micromillimeters.

485. Number of Fat Globules in Milk.—The number of fat globules in milk depends on their size and the percentage of fat. It is evident that no definite statement of the number can be made. There is a tendency, on the part of the globules, to diminish in size and increase in number as the period of lactation is prolonged. To avoid large numbers, it is convenient to give the

⁵⁸ Principles and Practice of Agricultural Analysis, 1906, 1 [2] : .

⁵⁹ Bureau of Chemistry, Bulletin 107 (revised), 1912 : 117.

⁶⁰ Wisconsin Agricultural Experiment Station, 6th Annual Report, 1889 : 64.

number of globules in 0.0001 cubic millimeter. This number may be found within wide limits depending on the individual, race, food and other local conditions to which the animal or herd is subjected. In general, in whole milk this number will be found between 140 and 250.

486. Method of Counting Globules.—The number of globules in milk is computed with the aid of the microscope. A convenient method is the one devised by Babcock.⁶¹ In carrying out this computation, capillary tubes, from two to three centimeters long and about one-tenth millimeter in internal diameter, are provided. The exact diameter of each tube, in at least three points, is determined by the micrometer attachment of the microscope, and from these measurements the mean diameter of the tube is calculated. This known, its cubic content for any given length is easily computed. Ten cubic centimeters of the milk are diluted with distilled water to half a liter and one end of a capillary tube dipped therein. The tube is quickly filled with diluted milk and each end is closed with a little wax to prevent evaporation. Several of these tubes being thus prepared, they are placed in a horizontal position on the stage of the microscope and covered with glycerol and a cover glass. The tubes are left at rest for some time until all the fat globules have attached themselves to the upper surfaces, in which position they are easily counted. The micrometer is so placed as to lie parallel with the tubes, and the number of globules, corresponding to each division of its scale, counted. The mean number of globules corresponding to each division of the micrometer scale is thus determined.

To compare the data obtained with each tube they are reduced to a common basis of the number of globules found in a length of fifty divisions of the micrometer scale in a tube having a diameter of 100 divisions, using the formula $N = \frac{10,000n}{d^2}$, in

which n = the number of globules found in the standard length of tube measured and d = the diameter of the tube. It is not difficult to actually count all the globules in a length of 50

⁶¹ New York (Geneva) Agricultural Experiment Station, Fourth Annual Report, 1886 : 267 et seq.

divisions of the scale, but the computation may also be made from the mean numbers found in a few divisions. The usual number of globules found in a length of 0.1 millimeter in a tube 0.1 millimeter in diameter, varies from 50 to 100.

Example.—The length of one division of the micrometer scale is 0.002 millimeter, and the internal diameter of the tube, 0.1 millimeter. The content of a tube, of a length of $0.002 \times 50 = 0.1$ millimeter, is therefore 0.0007854 cubic millimeter.

The cubic content of a tube 100 scale divisions in diameter and 50 in length is 0.0031416 cubic millimeter. The number of globules found in 50 divisions of the tubes used is 40. Then the number which would be contained in a tube of a diameter of 100 divisions of the micrometer scale and a length of 50 divisions thereof is $N = \frac{10,000n}{d^2} = \frac{400,000}{2,500} = 160$. Since the milk is

diluted 50 times, the actual number of globules corresponding to the volume given is 8,000. It is convenient to reduce the observations to some definite volume, *exempla gratia*, 0.0001 cubic centimeter. The equation for this in the above instance is $0.0031416 : 0.0001 = 8,000 : x$, whence $x = 223$, = number of fat globules in 0.0001 cubic millimeter.

In one cubic millimeter of milk there are therefore 2,230,000 fat globules, and in one cubic centimeter 2,230,000,000 globules. In a single drop of milk there are from one to two hundred million fat globules.⁶²

487. Method of Howard: An improved and simplified method depends upon the use of a Thoma-Zeiss blood counting cell and a disc eye-piece micrometer ruled in squares of about 0.5 millimeter.⁶³

The Thoma-Zeiss cell is so constructed that the layer of liquid under examination is just 0.1 millimeter in depth. It has an area ruled in squares but for milk globules this is useless since the globules rise to the top instead of settling to the bottom of the cell. In practice a magnification of about 200 gives good re-

⁶² Woll, Wisconsin Agricultural Experiment Station, Seventh Annual Report, 1890 : 239.

⁶³ Devised by B. J. Howard, unpublished.

sults. In preparing for the work it is first necessary to evaluate a certain area on the ruled eye-piece micrometer disc with the particular objective and eye-piece with which it is to be used. This is done by placing the eye-piece micrometer in place in the eye-piece and standardizing it by means of the rulings on the Thoma-Zeiss cell, or, better, by means of a stage micrometer. The exact area (a) in square millimeters represented by 10 of the small squares of the micrometer disc is determined once for all measurements.

In use, the counting cell and cover glass must be carefully polished so that when the cover is placed over the cell, a system of Newton's interference rings appear as a result of the close contact between the polished plate glass surfaces. Japanese lens paper is an excellent material with which to polish them.

Ten cubic centimeters of the milk to be tested is taken from the sample after thoroughly mixing and made up with distilled water to 250 cubic centimeters. A small drop of this is placed upon the central disc of the cell and the cover placed over it. A small drop should be used so as not to overflow the moat of the cell and interfere with the contact between cover and slide. After standing two or three minutes or until the fat globules have all come up into contact with the cover, the number in the 10 squares evaluated on the eye-piece disc of the micrometer are counted. Counts should be taken in two or more places on the slide and the average regarded as the true number (n). From the data thus obtained it is a simple matter to determine the number present in any desired volume of the original sample from the following formula:

$$N_v = n \left(\frac{dil \times v}{D \times a} \right)$$

Where N_v is the number sought in the volume (v) taken as a standard and for which 0.0001 cubic millimeter is a convenient amount. D is the depth of the cell in millimeters (usually 1/10 mm. on the Thoma-Zeiss cell), and dil represents the number of times the milk is diluted. Obviously the part of the equation within the parenthesis will always be a constant for a given

outfit and hence the calculation reduces finally to making one simple multiplication.

Substituting values in a certain case where 148 globules were counted in 10 squares having a determined area of 0.016 of a square millimeter we prove:

$$N_v = 148 \left(\frac{25 \times 0.0001}{0.1 \times 0.016} \right)$$

$$\text{or } N_v = 148 (1.562) = 231$$

Thus the only figure which will be likely to vary for different samples of milk when this outfit is used will be the actual number of globules counted (n).

488. Classification of Methods of Determining Fat in Milk.—

The fat, being the most valuable of the constituents of milk, is the subject of a number of analytical processes. An effort will be made here to classify these various methods and to illustrate each class with one or more typical processes. In general the methods may be divided into chemical and commercial, those of the first class being used for scientific and of the other for trade purposes. For normal milk, some of the trade methods have proved to be quite as accurate as the more chronokleptic analytical processes to which, in disputed cases, a final appeal must be taken. When the analyst is called upon to determine the fat in a large number of samples of milk some one of the trade methods may often be adopted with great advantage.

489. Dry Extraction Methods.—Among the oldest and most reliable methods of determining fat in milk, are included those processes based on the principle of drying the milk and extracting the fat from the residue by an appropriate solvent. The solvents generally employed are ether and petroleum spirit of low boiling point. The methods of drying are legion.

In extracting with ether, it must not be forgotten that other bodies than fat may pass into solution on the one hand and on the other the fats in any lecithin or nuclein which may be present may escape solution, at least in part. Perhaps petroleum spirit, boiling at from 45° to 60°, is the best solvent for fat, but it is

almost the universal custom in this and other countries to use ether.

490. Methods Based on use of Absorbents. (1) *The Asbestos Process*.—In this process it is directed to extract the residue from the determination of water by the asbestos method (481) with anhydrous pure ether until the fat is removed, evaporate the ether, dry the fat at 100° to constant weight. The fat may also be determined by difference, after drying the extracted cylinders at 100.°

(2) *Paper Coil Method*.—This is essentially the method proposed by Adams as modified by the author.⁶⁴ Coils made of thick filter paper are cut into strips 6.25 by 62.5 centimeters, thoroughly extracted with ether and alcohol, or the weight of the extract corrected by a constant obtained for the paper. If this latter method be used, a small amount of anhydrous sodium carbonate should be added. Paper free of matters soluble in ether is also to be had for this purpose. From a weighing bottle about five grams of milk are transferred to the coil by a pipette, taking care to keep dry the lower end of the coil. The coil, dry end down, is placed on a piece of glass, and dried at a temperature of boiling water for one hour, or better, dried in hydrogen at a temperature of boiling water, transferred to an extraction apparatus and extracted with absolute ether or petroleum spirit boiling at about 45°. The extracted fat is dried in hydrogen and weighed. Experience has shown that drying in hydrogen is not necessary. The fat may be conveniently dried in partial vacuo.

(3) *Evaporation on Sand*.—The sand should be pure, dry and of uniform size of grain. It may be held in a dish or tube. The dish may be made of tinfoil, so that it can be introduced with its contents into the extraction apparatus after the desiccation is complete. For this purpose, it is cut into fragments of convenient size after its contents have been poured into the extractor. The scissors used are washed with the solvent.

(4) *Evaporation on Kieselguhr*.—Dry kieselguhr (infusorial

⁶⁴ The Analyst, 1885, 10 : 46.

Division of Chemistry, Bulletin 13, Part I, 1887 : 85-86.

earth, Tripoli) may take the place of the sand as above noted. The manipulation is the same as with sand.

(5) *Evaporation on Plaster of Paris (Soxhlet Method)*,⁶⁵
(6) *On Pumice Stone*, (7) *On Powdered Glass*, (8) *On Chrysolite*.⁶⁶—The manipulation in these cases is conducted as with sand and no detailed description is required.

(9) *Evaporation on Organic Substances*.—These variations would fall under the general heading of drying on paper. The following materials have been used, *viz.*, sponge,⁶⁷ lint,⁶⁸ and wood pulp.⁶⁹ In these variations the principal precautions to be observed are to secure the organic material in a dry state and free of any matter soluble in the solvent used.

(10) *Dehydration with Anhydrous Copper Sulfate*.—In this process the water of the sample is absorbed by powdered anhydrous copper sulfate, the residual mass extracted and the butter fat obtained determined by saponification and titration.⁷⁰ In the manipulation about 20 grams of the anhydrous copper sulfate are placed in a mortar, a depression made therein in such a manner that 10 cubic centimeters of milk can be poured into it without wetting it through to the mortar. The water is soon absorbed and the mass is ground with a little dry sand and transferred to the extractor.

Petroleum spirit of low boiling point is used as a solvent, successive portions of about 15 cubic centimeters each being forced through the powdered mass under pressure. Two or three treatments with the petroleum are required. The residual butter fat, after the evaporation of the petroleum, is saponified with a measured portion, about 25 cubic centimeters, of semi-normal alcoholic potash lye. The residual alkali is determined by titration with semi-normal hydrochloric acid in the usual manner. From the data obtained is calculated the quantity of alkali employed in the

⁶⁵ Dingler; Polytechnisches Journal, 1879, **232** : 461.

⁶⁶ Macfarlane; The Analyst, 1893, **18** : 73.

⁶⁷ Duclaux; Le Lait, 1894 : 176.

⁶⁸ Abraham; The Analyst, 1884, **9** : 22.

⁶⁹ Ganttes; Zeitschrift für analytische Chemie, 1887, **26** : 677.

⁷⁰ Morse, Piggot and Burton; American Chemical Journal, 1887, **9** : 108 and 222.

saponification. The weight of butter fat extracted is then calculated on the assumption that 230 milligrams of potash are required to saponify one gram of the fat.

491. Gypsum Method for Sour Milk.—In sour milk, extraction of the dry residue with ether is attended with danger of securing a part of the free lactic acid in the extract. This may be avoided, at least in part, by making the milk neutral or slightly alkaline before desiccation. This method is illustrated by a variation of Soxhlet's method of drying on gypsum proposed by Kühn.⁷¹ The curdled milk is treated with potash lye of 40 per cent. strength until the reaction is slightly alkaline. For absorbing the sample before drying, a mixture is employed consisting of 25 grams of plaster of paris, four of precipitated carbonate of lime and two of acid potassium sulfate. To this mixture 10 grams of the milk, rendered alkaline as above noted, are added in a desiccating dish, the excess of moisture evaporated at 100°, the residual mass finely ground and extracted with ether for four hours. A little gypsum may be found in the solution, but in such small quantities as not to interfere seriously with the accuracy of the results obtained.

492. Estimation of Fat in Altered Milk.—In altered milk the lactose has usually undergone a fermentation affording considerable quantities of lactic acid. If such milks be treated by the extraction method for fat, the results will always be too high, because of the solubility of lactic acid in ether.

Vizern⁷² has proposed to avoid this error by first warming the soured milk for a few minutes to 40°, at which temperature the clabber is easily divided by vigorous shaking. Of the milk thus prepared, 30 grams are diluted with two or three volumes of water and poured onto a smooth and moistened filter. The vessel and filter are washed several times until the filtrate presents no further acid reaction. The filter and its contents are next placed in a vessel containing some fine washed sand. A small quantity of water is added, sufficient to form a paste. With a stirring rod, the filter is entirely broken up and the whole mass thoroughly

⁷¹ Chemiker-Zeitung Repertorium, 1889, 13 : 228.

⁷² Journal de Pharmacie et de Chemie, 1890, 5^e Série, 22 : 460.

mixed. Dried on the water-bath the material is subjected to extraction in the ordinary way. Several analyses made on fresh milk and on milk kept for several months show that almost identical results are obtained.

493. Comparison of Methods.—An immense amount of work has been done by analysts in comparing the various types of extraction methods outlined above.⁷³

While good results are obtained by all the methods when properly conducted, preference is given to the methods adopted by the Association of Official Chemists. As solvents, pure ether and petroleum spirit of low boiling point are preferred. The direct extraction gravimetric processes are important, since it is to these that all the other quicker and easier methods must appeal for the proof of their accuracy.

494. Wet Extraction Methods.—It has been found quite impracticable to extract the fat from milk by shaking it directly with the solvent. An emulsion is produced whereby the solvent itself becomes incorporated with the other constituents of the milk, and from which it is not separated easily even with the aid of whirling. The disturbing element which prevents the separation of the solvent is doubtless the colloid casein, since, when this is previously rendered soluble, the separation of the solvent holding the fat is easily accomplished.

The principle on which the methods of wet extraction are based is a simple one, *viz.*, to secure a complete or partial solution of the casein and subsequently to extract the fat with a solvent immiscible with water. The methods may be divided into three great classes, *viz.*, (1) those in which the solvent is evaporated from the whole of the extracted fat and the residual matters weighed; (2) processes in which an aliquot part of the fat solution is employed and the total fat calculated from the data secured; (3) the density of the fat solution is determined at a definite temperature and the percentage of fat corresponding thereto determined from tables or otherwise. Methods (1) and

⁷³ Richmond; *The Analyst*, 1892, 17: 48.

Division of Chemistry, *Bulletins* 28, 1890; 31, 1891; 35, 1892; 38, 1893; 43, 1894 and 46, 1896.

(2) are practically identical in principle and one or the other may be applied according to convenience or to local considerations. The methods may be further subdivided in respect of the reagents used to secure complete or partial solution of the casein, as, for instance, alkali or acid.⁷⁴

495. Solution in an Acid.—A good type of these processes is the method of Schmid.⁷⁵ Ten cubic centimeters of milk are placed in a test-tube of about five times that content, graduated to measure small volumes. An equal quantity of hydrochloric acid is added, the mixture shaken, boiled until it turns dark brown, and cooled quickly. The fat is extracted by shaking with 30 cubic centimeters of ether. After standing some time the ethereal solution separates and its volume is noted. An aliquot part of the solution is removed, the solvent evaporated, and the weight of fat in the whole determined by calculation.

The Schmid process has been improved by Stokes,⁷⁶ Hill⁷⁷ and Richmond.⁷⁸ The most important of these variations consists in weighing instead of measuring the milk employed, thus insuring grater accuracy. Dyer and Roberts affirm that the ether dissolves some of the caramel products formed on boiling condensed milk with hydrochloric acid, and that the data obtained in such cases by the process of Schmid are too high.⁷⁹

Since lactic acid is also slightly soluble in ether, sour milk should not be extracted with that solvent. In these cases petroleum spirit, or a mixture of petroleum and ether, as suggested by Pinette, may be used.⁸⁰ Another variation consists in extracting the fat with several portions of the solvent and evaporating all the extracts thus obtained to get the total fat. This method is perhaps the best of those in which the fat is extracted from the residual liquid after the decomposition of the casein by an acid.

⁷⁴ Division of Chemistry, Bulletin 28, 1890 : 31.

⁷⁵ Zeitschrift für analytische Chemie, 1888, 27 : 464.

⁷⁶ Chemical News, 1889, 60 : 214.

⁷⁷ The Analyst, 1891, 16 : 67.

⁷⁸ The Analyst, 1893, 18 : 53.

⁷⁹ The Analyst, 1892, 17 : 82.

⁸⁰ Chemiker-Zeitung, 1891, 15 : 1833.

and may be recommended as both reliable and typical within the limitations mentioned above.

496. Solution in an Alkali.—The casein of milk is not so readily dissolved in an alkali as in an acid, but the solution is sufficient to permit the extraction of the fat. Soda and potash lyes and ammonia are the alkaline bodies usually employed. To promote the separation of the emulsions, alcohol is added with advantage. The principle of the process rests on the observed power of an alkali to free the fat globules sufficiently to allow them to dissolve in ether or some other solvent. When the solvent has separated from the emulsion at first formed, the whole or a part of it is used for the determination of fat in a manner entirely analogous to that employed in the process with the acid solutions described above. There are many methods based on this principle, and some of the typical ones will be given below. Experience has shown that extraction from an alkaline solution is more troublesome and less perfect than from an acid and these alkaline methods are, therefore, not so much practiced now as they were formerly.

497. Method of Short.—Instead of measuring the volume of the separated fat, Short has proposed a method in which the casein is dissolved in an alkali and the fat at the same time saponified. The soap thus produced is decomposed by sulfuric acid and the volume of the separated fat acids noted. This volume represents 87 per cent. of the corresponding volume of fat.⁸¹

The solvent employed is a mixture of sodium and potassium hydroxids, containing in one liter 125 and 150 grams, respectively, of these alkalies. The sample of milk is mixed with half its volume of the reagent and placed in boiling water for two hours. By this treatment the casein is dissolved and the fat saponified. After cooling to about 60°, the soap is decomposed by the addition of equal parts of sulfuric and acetic acids. The tubes containing the mixture are again placed in boiling water for an hour and they are then filled with boiling water to within

⁸¹ Journal of Analytical Chemistry, 1888, 2 : 372.

Wisconsin Agricultural Experiment Station, Fifth Annual Report, 1888 : 125.

one inch of the top. The tubes may either be furnished with a graduation or the column of fat be measured by a scale.

498. Method of Thörner.—The process of Short is conducted by Thörner as follows:⁸²

Ten cubic centimeters of milk measured at 15° are saponified, in tubes fitting a centrifugal, by the addition of one and a half cubic centimeters of an alcoholic potash lye, containing 160 grams of potassium hydroxid per liter, or one cubic centimeter of an aqueous 50 per cent. soda lye. The saponification is hastened by setting the tubes in boiling water, where they remain for two minutes. The soap formed is decomposed with a strong acid, sulfuric preferred, the tubes placed in the centrifugal and whirled for four minutes, when the fat acids will be gathered in the narrow graduated part of the tube and the volume occupied thereby is noted after immersion in boiling water. Thörner's process is not followed in this country, but is used to a considerable extent in Germany.⁸³

499. Liebermann's Method.—In this method, 50 cubic centimeters of milk, at ordinary temperatures, are placed in a glass cylinder 25 centimeters high and about four and a half internal diameter; five cubic centimeters of potash lye of 1.27 specific gravity are added, the cylinder closed with a well fitting cork stopper and thoroughly shaken.⁸⁴ After shaking, 50 cubic centimeters of petroleum spirit, boiling point about 60°, are added. The cylinder is again stoppered and vigorously shaken until an emulsion is formed. To this emulsion 50 cubic centimeters of alcohol of 95 per cent. strength are added, and the whole again thoroughly shaken. After four or five minutes the petroleum spirit, containing the fat, separates. In order to insure an absolute separation of the fat, however, the shaking may be repeated three or four times for about one-quarter minute, waiting each time between the shakings until the spirit separates.

Of the separated petroleum spirit 20 cubic centimeters are placed in a small weighed flask. The use of the flask is recom-

⁸² *Chemisches Central-Blatt*, 1892, 2 : 492.

⁸³ *Chemiker-Zeitung*, 1894, 18 : 1816; 1895, 19 : 348.

⁸⁴ *Zeitschrift für analytische Chemie*, 1893, 32 : 170.

mended on account of the ease with which the petroleum spirit can be evaporated without danger of loss of fat. Instead of the flask a weighed beaker or other weighed dish may be employed.

The petroleum spirit is carefully evaporated on a water-bath and the residue dried at 110° to 120° for one hour. The weight found multiplied by five gives the content of fat in 100 cubic centimeters of the milk. The percentage by weight can then be calculated by taking into consideration the specific gravity of the milk employed.

The results obtained by this method agree well with those obtained by the paper coil method, when petroleum spirit instead of sulfuric ether is used as the solvent for the fat. Sulfuric ether, however, gives an apparently higher content of fat because of the solution of other bodies not fat present in the milk.

500. Roese-Gottlieb Method for Fat in Condensed and Evaporated Milks.—The slightly modified method which gives excellent results is as follows:⁵⁵

Make a 20 per cent. water solution of the homogeneous sample. Of this solution measure 10 cubic centimeters or about 10 grams in a Rohrig tube⁵⁶ or a glass cylinder three-fourths of an inch in diameter and about 14 inches high, to which a narrow siphon can be fitted or other similar apparatus, add one cubic centimeter of concentrated ammonia (two cubic centimeters if the material is sour) and mix thoroughly with the milk. Add 10 cubic centimeters of 92 per cent. alcohol and shake well, then 25 cubic centimeters of ethyl ether, shake vigorously for two or three minutes, add 25 cubic centimeters of petroleum ether (boiling point below 70°) and shake again. After standing 15 minutes, or until the upper liquid is clear and its lower level constant, should the dividing line not be distinct, add 0.3 gram of powdered salt and shake gently until the salt is dissolved. Take the upper and lower readings of the ethereal liquid and draw off as much as possible (usually 0.5 to 0.8 cubic centimeter is left) into a

⁵⁵ Landwirtschaftliche Versuchs Stationen, 1892, 40 : 1-27.

⁵⁶ Zeitschrift für Untersuchungs Nahrungs und Genussmittel, 1905, 9 : 531.

weighed flask through a diminutive filter which must then be washed with a small quantity of the mixed solvents (1:1).

Read the volume of ethereal liquid left in the tube and deduct from the total volume. Evaporate the drawn off and filtered liquid *slowly* in a hood and then dry in a boiling water oven one hour or less at a time, until loss of weight ceases. Extract the liquid remaining in the tube in the same manner as before, but this time for economy the volume of the ethers used may be reduced to from 10 to 15 cubic centimeters, using of each equal volumes in each case. It is preferable to evaporate and weigh this extract separately, as a check upon the work. The solvents must be tested for residue upon evaporation, and, if any is found, the result of the analysis must be corrected therefor.

The purity of the dried and weighed fats should always be proved, by dissolving in a little petroleum ether, should a residue be found, which can only happen when a trace of the aqueous liquid has accidentally passed the filter, it must be washed back into the flask, dried, and its weight deducted from that of the crude fat.

This method is equally applicable to sweetened and unsweetened condensed milks, to ice cream, cream, milk, skim milk, butter-milk and whey. With substances of low fat content the second extraction may be omitted, in which case the weight of the fat actually obtained must be increased to correspond to the entire volume of ethereal liquid measured in the tube.

In the application of this method to ice cream about 10 grams of the melted well mixed sample is used.

With milk powder a preliminary treatment of the sample is necessary. About one gram of well mixed sample is placed in a small beaker, treated with nine cubic centimeters of water and one cubic centimeter of concentrated ammonia, heated on steam-bath, until an emulsion is formed, cooled, transferred to a Rohrig tube and treated as above described.

501. Densimetric Methods.—Instead of evaporating the separated fat solution and weighing the residue, its density may be determined and the percentage of fat dissolved therein obtained by calculation, or more conveniently from tables. The typical

method of this kind is due to Soxhlet, and until the introduction of modern rapid volumetric processes, it was used perhaps more

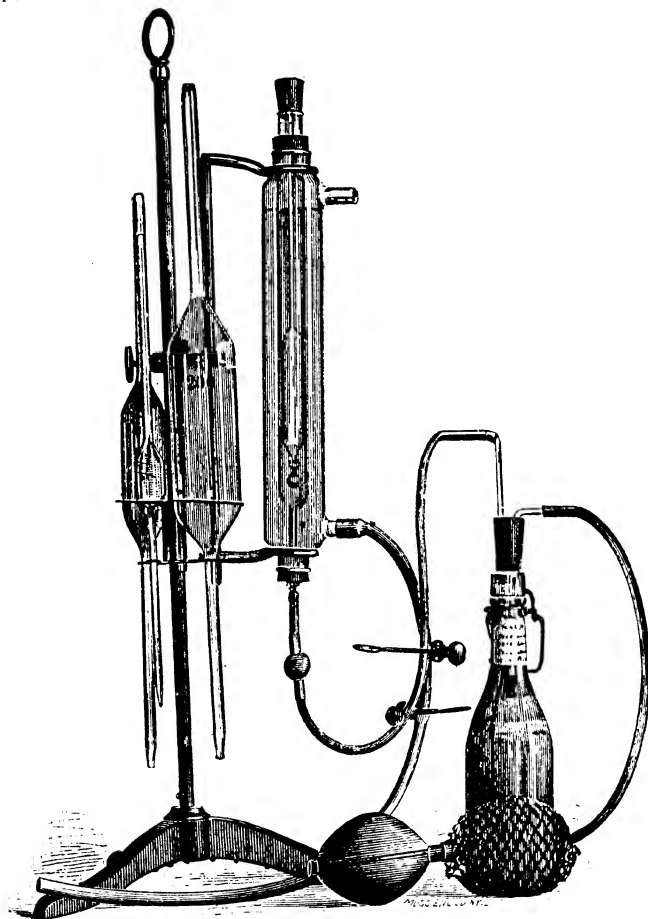


Fig. 101.—Densimetric Fat Apparatus.

extensively than any other proceeding for the determination of fat in milk.⁸⁷ The reagents employed in the process are ether

⁸⁷ *Zeitschrift des Landwirthschaftlichen Vereins in Bayern*, 1880, 70 : 659.

Zeitschrift für analytische Chemie, 1881, 20 : 452.

saturated with water and a potash lye containing 400 grams of potash in a liter. The principle of the process is based on the assumption that a milk made alkaline with potash will give up all its fat when shaken with ether and the quantity of fat in solution can be determined by ascertaining the specific gravity of the ethereal solution.

TABLE FOR CALCULATING PER CENT. OF FAT IN MILK BY DENSIMETRIC METHOD OF SOXHLET.

Reading of oleometer.	Per cent. fat in milk.	Reading of oleometer.	Per cent. fat in milk.
43	2.07	55	3.49
44	2.18	56	3.63
45	2.30	57	3.75
46	2.40	58	3.90
47	2.52	59	4.03
48	2.64	60	4.18
49	2.76	61	4.32
50	2.88	62	4.47
51	3.00	63	4.63
52	3.12	64	4.79
53	3.25	65	4.95
54	3.37	66	5.12

The apparatus is arranged as shown in Fig. 101, whereby it is easy to drive the ethereal fat solution into the measuring vessel by means of the bellows shown. In the bottle, seen at the right of the engraving are placed 200 cubic centimeters of milk, 10 of the potash lye and 60 of the aqueous ether. The milk and potash are first added and well shaken, the ether then added, and the contents of the bottle are shaken until a homogeneous emulsion is formed. The bottle is then set aside for the separation of the ethereal solution, which is promoted by gently jarring it from time to time. When the chief part of the solution has separated, a sufficient quantity of it is driven over into the measuring apparatus, by means of the air bulbs, to float the hydrometer contained in the inner cylinder. After a few moments the scale of the oleometer is read and the percentage of fat calculated from the table. All the measurements are made at a temperature of 17°.5. The temperature is preserved constant by filling the outer cylinder of the apparatus with water. If the room be warmer

than $17^{\circ}.5$, the water added should be at a temperature slightly below that and *vice versa*. The oleometer carries a thermometer which indicates the moment when the reading is to be made.

The scale of the oleometer is graduated arbitrarily from 43 to 66, corresponding to the specific gravities of 0.743 and 0.766, respectively, or to corresponding fat contents of 2.07 and 5.12 per cent. in the milk, a range which covers most normal milks.

In the use of the table the per cents. corresponding to parts of an oleometer division can be easily calculated.

502. Application of the Densimetric Method.—Soxhlet's method, as outlined above, with many modifications, has been extensively used in Europe and to a limited degree in this country, and the results obtained are in general satisfactory, when the sample is a mixed one from a large number of cows and of average composition.

The author has shown that the process is not applicable to abnormal milk and often not to milk derived from one animal alone.⁸⁸

The chief difficulty is found in securing a separation of the emulsion. This trouble can usually be readily overcome by whirling. Any centrifugal machine, which can receive the bottle in which the emulsion is made, may be employed for that purpose.

Since the introduction of more modern and convenient methods of fat determination, the densimetric method has fallen into disuse and perhaps is no longer practiced in this country. It is valuable now chiefly from the fact that many of the recorded analyses of milk fat were made by it, and also for its typical character in representing methods of analysis of fat in milk based on the density of ethereal solutions.

503. The Lactobutyrometer.—A typical instrument for measuring the volume of fat in a milk is known as Marchand's lactobutyrometer. It is based on the observation that ether will dissolve the fat from milk when the casein is wholly or partly dissolved by an alkali, and further, that the fat in an impure form can be separated from its ethereal solution by the action of

⁸⁸ Bulletin 13, Part 1, Division of Chemistry, 1887 : 92.

alcohol. Experience has shown that all the fat is not separated from the ethereal solution by this process, and also that the part separated is a saturated solution in ether. The method cannot be rigorously placed in the two classes given above, but being volumetric demands consideration here chiefly because of its historical interest.⁸⁹

The instrument employed by Marchand is a tube about 30 centimeters long and 12 in diameter, closed at one end and marked in three portions of 10 cubic centimeters each. The upper part is divided in tenths of a cubic centimeter. The superior divisions are subdivided so that the readings can be made to hundredths of a cubic centimeter.

The tube is filled with milk to the first mark and two or three drops of a 25 per cent. solution of soda lye added thereto. Ether is poured in to the second mark, the tube closed and vigorously shaken. Alcohol of about 90 per cent. strength is added to the upper mark, the tube closed, shaken and allowed to stand in a vertical position, with occasional jolting, until the separation of the liquids is complete. In order to promote the separation the tube is placed in a cylinder containing water at 40°.

When the separation is complete the milk serum is found at the bottom, the mixture of alcohol and ether in the middle and the fat at the top. The mixture of ether and alcohol contains 0.126 gram of fat, and each cubic centimeter of the separated ether fat 0.233 gram of fat. The total volume of the separated fat, multiplied by 0.233, and the product increased by 0.126, will give the weight of fat in the 10 cubic centimeters of milk employed.

Example.—Milk used, 10 cubic centimeters of 1.032 specific gravity = 10.32 grams. The observed volume of the saturated ether fat solution is two cubic centimeters. Then the weight of fat is $2 \times 0.233 + 0.126 = 0.592$ gram. The percentage of fat in the sample is $0.592 \times 100 \div 10.32 = 5.74$.

In the apparatus the upper division of the graduation is marked 12.6, because this represents the quantity of fat which remains in the ether alcohol mixture for one liter of milk. From

⁸⁹ Becke, *Die Milchprüfungs-Methoden*, 1882 : 66.

this point the graduation is extended downward to 95, which, for 10 cubic centimeters of milk, represents 0.95 gram. After the fat has separated, enough 95 per cent. alcohol is added to bring the upper surface exactly to the graduation 12.6. The number of grams per liter of milk is then read directly from the scale.

In respect of applicability, the observation made regarding Soxhlet's densimetric method may be repeated.

In practical work in this country the lactobutyrometer is no longer used, but many of the recorded determinations of fat in milk have been made by this method.

504. Volumetric Methods.—For practical purposes the volumetric methods of estimating fat in milk have entirely superseded all the other processes. It has been found that the fat readily separates in a pure state from the other constituents of milk whenever the casein is rendered completely soluble; whereas no process has yet been devised whereby the fat can be easily separated in a pure state from milk which has not been treated with some reagent capable of effecting a solution of the casein. The volumetric methods may be divided into two classes, *viz.*, (1) those in which the fat is separated by the simple action of gravity, and (2) those in which the natural action of gravity is supplanted by centrifugal motion. Each of these classes embraces a large number of variations and some of the typical ones will be described in the following paragraphs. As solvents for the casein a large number of reagents has been used, including alkalies and single and mixed acids. In practice, preference is given to the least complex and most easily prepared solvents.

505. Method of Patrick.—A typical illustration of the method of collecting the fat after solution of the casein, without the aid of whirling, is found in the process devised by Patrick.⁹⁰

The solvent employed is a mixture of acetic, sulfuric and hydrochloric acids, saturated with sodium sulfate, in the respective volumetric proportions of nine, five and two. The separation is accomplished in a large test-tube drawn out near the top into

⁹⁰ Bulletin 8, Iowa Agricultural Experiment Station, 1890 : 295.

a constricted neck which is graduated to measure the volume of the separated fat or to give direct percentage results.

The tube should have a content of about 25 cubic centimeters below the upper mark on the neck. In use 10.4 cubic centimeters of milk and a sufficient quantity of the mixed acids to fill it nearly to the upper mark are placed in the tube, together with a piece of pumice stone, and the mixture boiled. On cooling below 100° , the fat will separate and the volume thereof may be measured in the constricted portion of the tube. The volume of the fat may be converted into weight on multiplying by 0.88 at 60° , or more conveniently the percentage of fat be taken from a table. In practice, the tube is filled with the milk and acid mixture nearly up to the neck, its contents well mixed and additional acid mixture added until the liquid is raised in the tube above the neck. After mixing a second time, the contents are boiled for five minutes and the fat allowed to collect in the expanded part of the tube above the neck. When the fat has collected, the mixture is boiled gently a second time for a few minutes. By this treatment the fat is mixed with the upper portions of the acid liquid and clarified. The clearing of the fat may be hastened by sprinkling over it a little effloresced sodium sulfate. The fat is brought into the graduated neck by opening a small orifice in the belly of the tube, which is closed by means of a rubber band. When the temperature has reached 60° , the space occupied by the fat is noted and the numbers obtained express the percentage of fat in the sample.

This process is illustrative of the principle of analysis, but is no longer used in analytical determinations.

506. The Lactocrite.—One of the earliest methods for fat estimation in milk, depending on the solution of the casein and the collection of the fat by means of whirling, is based on the use of a centrifugal machine known as the lactocrite. This apparatus is modeled very like the machine usually employed for creamery work,⁹¹ and at one time was extensively used, but it has now given place to less troublesome and expensive machines. The acid mixture for freeing the fat of casein is composed of glacial

⁹¹ Dingler's Polytechnisches Journal, 1886, 261 : 219.

acetic acid carrying five per cent. of sulfuric. The samples of milk are heated with the acid mixture in test-tubes provided with stoppers and short glass tubes to return the condensation products. The hot mixture is poured into a small metallic cylindrical cup holding about three cubic centimeters. This cup fits by means of an accurately ground shoulder on a metal casing, carrying inside a heavy glass graduated tube of small internal diameter. The excess of the milk mixture escapes through a small aperture in the metallic screw cap of the metal holder. The metal holder is cut away on both sides in order to expose the graduations on the glass tube. The glass tube is held water tight by means of perforated elastic washers. Thus prepared the tubes are inserted in the radial holes of a revolving steel disk previously heated to a temperature of 60°. The whirling is accomplished in a few minutes by imparting to the steel disk a speed of about 6,000 revolutions per minute. At the end of this operation the fat is found in a clear column in the small glass tube and the number of the divisions it occupies in this tube is noted. Each division of the scale represents one-tenth per cent. of fat.

This apparatus is capable of giving accurate results when all its parts are in good working order. The chief difficulty which its use has presented is in keeping the joints in the glass metal tube tight.

This description of the apparatus is given to secure an illustration of the principle involved, a principle which has been worked out in later times into some of the most rapid and practical processes of estimating fat in milk.

507. Modification of Lindström.—Many modifications have been proposed for conducting the determination of fat by means of the lactocrite, but they do not involve any new principle and are of doubtful merit. In the modification suggested by Lindström, which has attained quite an extended practical application, the solvent mixture is composed of lactic and sulfuric acids and the butyrometer tubes are so changed as to permit the collection of the fat in the graduated neck after whirling, by means of adding water. The apparatus is also adjusted to secure the con-

*gelation of the fat column before its volume is noted.*⁹² The analyst can read the fat volume at his leisure when it is in the solid state and is not confused by changes of volume during the observation. The best acid mixture has been found to be composed of 100 volumes of lactic, an equal amount of acetic and 15 volumes of sulfuric acids.

508. The Babcock Method.—Among the many quick volumetric methods which have been proposed for the determination of fat in milk, none has secured so wide an application as that suggested by Babcock.⁹³

The chief point of advantage in the use of this method is found in effecting the solution of the casein by means of sulfuric acid of about 1.83 specific gravity. By this reagent the casein is dissolved in a few moments without the aid of any other heat than that generated by mixing the milk with the reagent. The bottle in which the separation is made is shown in Fig. 102. The graduations on the neck are based on the use of 18 grams of milk. To avoid the trouble of weighing, the milk is measured from a pipette graduated to deliver 18 grams of milk of the usual specific gravity. While it is true that normal milk may vary somewhat in its density, it has been found that a pipette marked at 17.6 cubic centimeters delivers a weight which can be safely assumed to vary but slightly from the one desired. The graduated bottle holds easily 35 cubic centimeters of liquid in its expanded portion and the volume of milk just noted is mixed with an equal volume of sulfuric acid, conveniently measured from the lip cylinder shown in the figure. The complete mixture of the milk and acid is effected by gently rotating the bottle until its contents are homogeneous. The final color of the mixture varies from dark brown to black.

While still hot, the bottles are placed in a centrifugal machine and whirled for at least five minutes. The most convenient machine, where it is available, is the one driven by a jet of steam. The revolutions of the centrifugal should be at least 700 per minute for a 20 inch and 1,200 for a 12 inch wheel. After five

⁹² *Milch Zeitung*, 1892, 21 : 496.

⁹³ Bulletin 24, Wisconsin Agricultural Experiment Station, 1890.

minutes the bottles are removed and filled to the upper mark or nearly so with hot water, replaced in the machine and whirled for at least one minute. The fat will then be found in a clearly defined column in the graduated neck of the bottle. In reading the scale, the extreme limits between the lowest point marked by the lower meniscus and the highest point marked by the edge of the

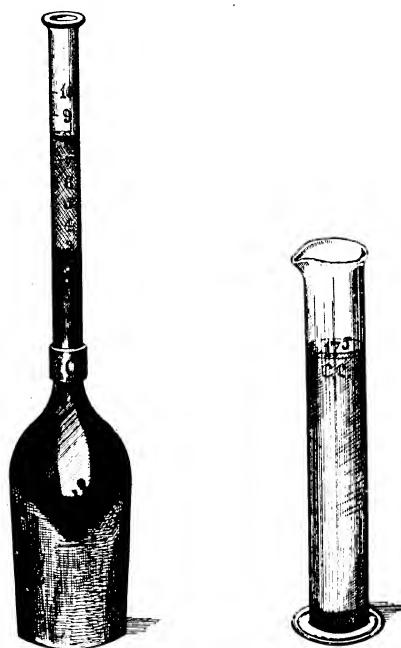


Fig. 102.—Babcock's Butyrometer and Acid Measure.

upper meniscus are to be regarded as the termini of the fat column.

In testing cream by the Babcock process, it may either be diluted until the column of fat secured is contained in the graduated part of the neck or specially graduated bottles may be used.

Condensed Milk.—In applying the Babcock test to condensed milk, it is necessary to weigh the sample and to use only about

eight grams.⁹⁴ This quantity is placed in the bottle and dissolved in 10 cubic centimeters of water and the analysis completed as above. The reading noted is multiplied by 18 and divided by the weight of the sample taken.

Skim Milk.—In determining the fat in skim milk and whey, it is desirable to use a bottle of double the usual capacity, but with the same graduation on the neck. The percentage of fat noted is divided by two.

Cheese.—Five grams are a convenient quantity of cheese to employ. To this quantity in the bottle are added 15 cubic centimeters of hot water and the flask gently shaken and warmed until the cheese is softened. The treatment with acid and whirling are the same as described above. The noted reading is multiplied by 18 and divided by five.

509. Solution in Amyl Alcohol and Hydrochloric and Sulfuric Acids.—Leffmann and Beam have proposed to aid the solution of the casein in sulfuric acid by the previous addition to the milk of a mixture of equal volumes of amyl alcohol and hydrochloric acid.⁹⁵ In this process the same graduated flasks may be used as in the Babcock process, or a special flask may be employed. In this case the graduation of the neck is for 15 cubic centimeters of milk, and each one and a half cubic centimeters is divided into 86 parts. The quantity of milk noted is placed in the flask, together with three cubic centimeters of the mixture of amyl alcohol and hydrochloric acid, and well shaken. To the mixture, sulfuric acid of 1.83 specific gravity is added until the belly of the flask is nearly full and the contents well mixed by shaking. When the casein is dissolved, the addition of the sulfuric acid is continued until the flask is filled to the upper mark and again the contents mixed. It is well to close the mouth of the flask with a stopper while shaking. The bottle is placed in a centrifugal and whirled for a few moments, when the fat is collected in the graduated neck and its volume noted.

The process is also known in this country as the Beimling

⁹⁴ Bulletin 31, Wisconsin Agricultural Experiment Station, 1892 : 11.

⁹⁵ The Analyst, 1892, 17 : 83.

method.⁹⁶ The fat separated in the above process is probably mixed with a little fusel oil, and therefore it is advisable to use the specially graduated bottle instead of one marked in absolute volumes.⁹⁷

The method, when conducted according to the details found in the papers cited, gives accurate results, but is somewhat more complicated than the Babcock process and is not now used to any great extent in analytical work.

510. Method of Gerber.—The method proposed by Gerber for

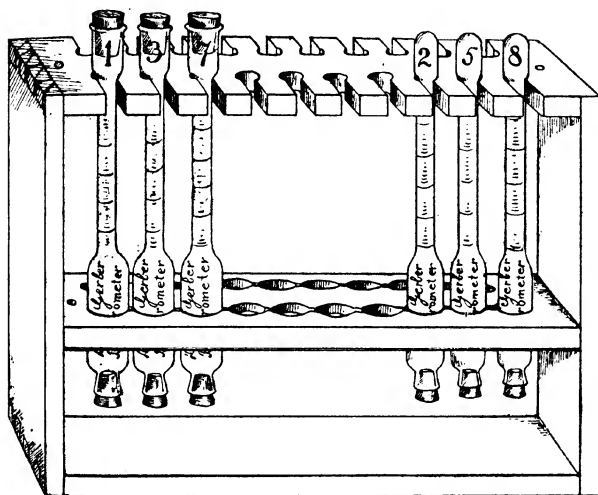


Fig. 103.—Gerber's Butyrometers.

estimating fat in milk is based on the processes of Babcock, Beimling and Beam already described. The tubes in which the decomposition of the milk and the measurement of the fat are accomplished are of two kinds, one open at only one end for milk and the other open at both ends for cheese. They are closed during the separation by rubber stoppers.⁹⁸

The apparatus have been greatly improved and simplified since

⁹⁶ Bulletin 21, Vermont Agricultural Experiment Station, 1890 : 46.

⁹⁷ The Analyst, 1892, 17 : 145 ; 1893, 18 : 130 ; 1894, 19 : 62.

⁹⁸ Chemiker-Zeitung, 1892, 16 : 1839.

the first description of them was published and have come into extensive use in Europe and to a limited extent in this country.⁹⁹

The butyrometer tubes are made of various sizes and shapes, but the most convenient are those noted above as shown in Fig. 103.

Before adding the strong sulfuric acid, one cubic centimeter of amyl alcohol is mixed with the milk in the butyrometer. This admixture serves to clarify the fat and render the reading more easy.

The centrifugal is run by hand, and the required speed of rotation is given it by means of a cord wrapped spirally about its axis, as shown in Fig. 104. The cord in the new machines is replaced by a leather strap working on a ratchet.

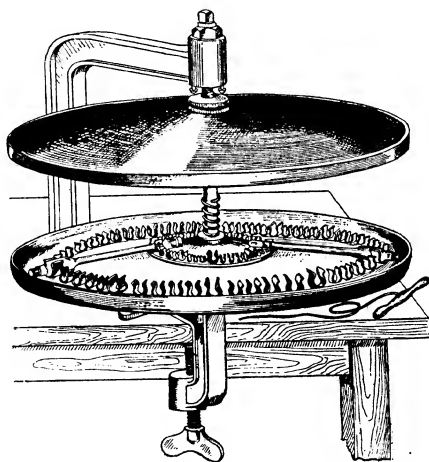


Fig. 104.—Gerber's Centrifugal.

The process is more speedy than that of Babcock, and the results have been shown by a large experience to be reliable and accurate.

The sulfuric acid employed is of 1.825 to 1.830 specific gravity. There is no danger of loss by the formation of volatile ethers where the quantity of amyl alcohol used does not exceed one

⁹⁹ Chemiker-Zeitung, 1895, 19 : 348; 1894, 18 : 1816.

cubic centimeter. In a comparison of the respective merits of the methods of Babcock, Thörner and Gerber, made by Hausmann, the first place is awarded to the Gerber process.¹ Long experience in this country, however, has shown the decided superiority of the Babcock process. In Fig. 103, the butyrometers marked 2, 5 and 8 are for milk, and those numbered 1, 3 and 7 are for cream and cheese. In conducting the analysis, 10 cubic centimeters of the sulfuric acid are placed in the butyrometer with one cubic centimeter of the amyl alcohol. When mixed, 11 cubic centimeters of the milk are added and the contents of the tube well mixed, the tube stoppered and placed in the centrifugal. The larger tubes, open at both ends, require double the quantities of the reagents mentioned. The measurements are made at about 15°.

Minute directions for conducting the analyses with milk, skim milk, buttermilk, cream, condensed milk, cheese and butter accompany each apparatus.

PROTEIN BODIES IN MILK.

511. Kinds of Protein Bodies in Milk.—The protein bodies in milk are all found in at least partial solution. Some authorities state that a portion of the casein is present in the form of fine particles suspended after the manner of the fat globules.² The number and kind of protein bodies are not known with definiteness. Among those which are known with certainty are casein, albumin, peptone and fibrin. The latter body was discovered in milk by Babcock.³ Lactoglobulin and lactoprotein are also names given to imperfectly known protein bodies in milk. Lactoprotein is not precipitated either by acids or by heat and is therefore probably a peptone. By far the greater part of the protein matters in milk is casein. Casein has been called caseinogen by Halli-

¹ *Chemiker-Zeitung*, 1895, 19 : 348.

² *Comptes rendus*, 1888, 107 : 772.

Hoppe-Seyler, *Handbuch der Physiologisch- und Pathologisch-Chemischen Analyse*, 1909 [8] : 718.

³ *Proceedings of the Society for the Promotion of Agricultural Science*, Ninth Annual Meeting, 1888 : 13.

burton,⁴ and paracasein by Schulze and Röse.⁵ Casein has intimate relations to the mineral matters in milk, and is probably itself made up of several protein bodies of slightly differing properties. In general all that class of protein matter contained in milk which is precipitated by rennet or a weak acid, or spontaneously on the development of lactic acid, is designated by the term casein, while the albumins and peptones in similar conditions remain in solution. Casein contains phosphorus, presumably as nuclein. Fibrin is recognized in milk by the reactions it gives with hydrogen peroxid or gum guaiacum. The decomposition of hydrogen peroxid is not a certain test for fibrin, inasmuch as pus and many other bodies will produce the same effect. If the milk decompose hydrogen peroxid, however, before and not after boiling, an additional proof of the presence of fibrin is obtained, since boiled fibrin does not act on the reagent.⁶ The gum guaiacum test is applied by dipping a strip of filter paper into the milk and drying. The solution of gum guaiacum is applied to the dried paper and the presence of fibrin is recognized by the blue color which is produced. The fibrin is probably changed into some other protein during the ripening of cream in which the fibrin is chiefly found. The albumin in milk is coagulated by boiling, while the casein remains practically unaffected when subjected to that temperature.

212. Estimation of Total Protein Matter.—The total protein matter in milk is determined by any of the general methods applicable to the estimation of total nitrogen, but the moist combustion method is by far the most convenient. From the total nitrogen, that which represents ammonia or other nonprotein nitrogenous bodies, is to be deducted and the remainder multiplied by an appropriate factor. Practically all the nitrogen obtained is derived from the protein matters and, as a rule, no correction is necessary. The factors employed for calculating the weight of protein matter from the nitrogen obtained vary from 6.25 to 7.04.

⁴ Journal of Physiology, 1890, 11 457.

⁵ Die Landwirthschaftlichen Versuchs-Stationen, 1895, 31 : 131.

⁶ Wisconsin Agricultural Experiment Station, Sixth Annual Report, 1889 : 64.

It is desirable that additional investigations be made to determine the magnitude of this factor. It is suggested that provisionally the factor 6.38 be used. In the method adopted by the Association of Official Agricultural Chemists it is directed that about five grams of milk be placed in the oxidizing flask and treated without previous evaporation exactly as described for the estimation of total nitrogen in the absence of nitrates. The nitrogen obtained is multiplied by 6.38 to get the total protein matter.⁷ In order to prevent the too great dilution of the sulfuric acid, the milk may be evaporated to dryness or nearly so before oxidation. This is conveniently done by placing the milk first in the oxidizing flask, connecting this with the vacuum service and placing the flask in hot water. The aqueous contents of the milk are quickly given off at a temperature not exceeding 85°, and the time required is only a few minutes.

The milk may also be dried in dishes made of thin glass or tinfoil and, after desiccation, introduced with the fragments of the dishes into the oxidizing flask.

The preliminary drying in the oxidizing flask is recommended as the best.

Söldner oxidizes the nitrogen in human milk by boiling 10 cubic centimeters thereof for three hours with 25 of sulfuric acid, 50 milligrams of copper oxid and three drops of a four per cent. platonic chlorid solution, and, after distilling the ammonia, uses the factor 6.39 for calculating the protein matter. According to this author human milk is much less rich in nitrogenous constituents than is generally supposed, containing not more than one and a half per cent. thereof in average samples collected at least a month after parturition.⁸

513. Precipitation of Total Proteins with Copper Sulfate.—This method of throwing out the total proteins of milk is due to Ritthausen.⁹ The proteins and fat are precipitated together by the addition of a measured volume of copper sulfate solution,

⁷ Bulletin 46, Division of Chemistry, 1895 : 36.

Bureau of Chemistry, Bulletin 107 (revised), 1912 : 117.

⁸ Zeitschrift für Biologie, 1896, 33, Neue Folge, 15 : 43.

⁹ Journal für Praktische Chemie, 1877, Neue Folge, 15 : 329.

containing 63.92 grams of the crystallized salt in one liter. The process, as modified by Pfeiffer, is conducted as follows:¹⁰

Ten grams of milk are diluted with 10 times that much water, five cubic centimeters of the copper sulfate solution added and then soda lye solution drop by drop until the copper is just precipitated. This is determined by testing a few drops of the filtrate with soda lye, which, when the copper is precipitated, will give neither a turbidity nor a blue color.

The mixture is poured into a dry tared filter, the precipitate washed with hot water, dried to constant weight and weighed. The fat is removed from the dry pulverized mass by extraction with ether and the residue dried and weighed.

The quantity of copper oxyhydrate contained in the precipitate is calculated from the quantity of the copper solution used and amounts to 0.2026 gram for five cubic centimeters. The casein thus prepared contains not only the copper compound named, but also some of the sodium sulfate formed on the addition of the soda lye and other mineral salts present in the milk and from which it is quite impossible to completely free it. There are also many other objections to the process, and the product is of such a nature as to render the data obtained by the method very doubtful.

This method is chiefly valuable on account of its historical interest. Not only are the drying and weighing of the precipitate rendered unnecessary by the modern methods of determining nitrogen, but there are numerous sources of error which seem to throw doubt on the accuracy of the results. The copper hydroxid does not lose all its water even on drying at 125°.¹¹ The method therefore can only be recommended for practical purposes when all the tedious processes of drying, extracting and calculating the quantity of copper oxid are abandoned and the moist washed precipitate used directly for the determination of nitrogen.

514. Protein Bodies by Ammonium Sulfate.—All the protein bodies except peptones are precipitated from milk on saturation

¹⁰ Zeitschrift für Biologie, 1896, **33**, Neue Folge, **15** : 55.

¹¹ Stenberg, Zeitschrift für Physiologische Chemie, 1889, **13** : 138.

with ammonium sulfate. This method has little analytical value because of the presence of nitrogenous salt in the precipitate. Zinc sulfate may be substituted for the ammonium salt and thus a determination of protein matter other than peptone be obtained. This result subtracted from the total protein nitrogen gives that due to peptone.

515. Total Protein Matter by Tannic Acid.—For the determination of the total protein matter in milk Sebelien uses the following process.¹² From three to five grams are diluted with three or four volumes of water, a few drops of a saline solution added (sodium phosphate, sodium chlorid, magnesium sulfate, *et similia*), and the protein bodies thrown out with an excess of tannic acid solution. The precipitate is washed with an excess of the precipitant and the nitrogen therein determined and multiplied by 6.37.

516. Separation by Porous Porcelain.—In addition to the methods already described for separating the soluble and suspended protein bodies in milk, and which may be used also for koumiss, the following should also be mentioned as of especial worth:

Separation by Filtration through Porous Porcelain.—A purely physical method, and one which is to be recommended by reason of the absence of any chemical action upon the different protein matters, it that proposed by Lehmann, depending upon the principle that when milk is forced through porous porcelain the albumin passes through together with the milk, sugar and other soluble constituents as a clear filtrate, while the casein and fat are perfectly retained.¹³

By this method it is quite certain that the albumen and other perfectly soluble proteins of milk may be obtained in the purest form.

Separation by Precipitation with Alum.—Probably the best chemical method of separating the two classes of protein matters

¹² Zeitschrift für Physiologische Chemie, 1889, **13** : 137.

¹³ Pflüger; Archiv für die gesammte Physiologie, 1894, **56** : 558.

is that proposed by Schlossmann, which is effected by means of precipitating the casein with a solution of alum.¹⁴

The principle of this separation rests upon the fact that a solution of potash alum, when added to milk diluted with four or five times its volume of water, will completely separate the casein without affecting the albumin or globulin. The operation is conducted as follows:

Ten cubic centimeters of the milk are diluted with from three to five times that quantity of water and warmed to a temperature of about 40°. One cubic centimeter of a concentrated solution of potash alum is added, the mixture well stirred and the coagula which are formed allowed to subside. If the coagulation of the casein does not take place promptly, a small addition of the alum solution is made, usually not exceeding half a cubic centimeter, until the precipitation is complete. The temperature during the process should be kept as nearly as possible at 40°. After a few minutes, the mixture is poured upon a filter and the filtrate, if not perfectly clear, is poured back until it is secured free of turbidity. In difficult cases the filtration may be promoted by the addition of some common salt or calcium phosphate, the latter acting mechanically in holding back the fine particles of casein. The precipitate is washed with water at a temperature of 40°, and afterwards with alcohol, not allowing the alcohol wash water to flow into the filtrate. When the water has been chiefly removed from the precipitate by washing with alcohol, the fat of the precipitated casein is removed with ether and the residue used for the determination of nitrogen in the usual way. The albumin is removed from the filtrate by a tannin solution in the manner already described. If it be desired to separate the albumin and globulin, the methods described in paragraph 524, 525 may be used.

517. Mercurial Method.—A volumetric method for determining the total protein matter in milk has lately been proposed by Deniges.¹⁵ It is based upon the observation that in the precipitation of protein matter by mercury salts, a definite quantity of

¹⁴ Hoppe-Seyler, *Zeitschrift für Physiologische Chemie*, 1896-97, **22** : 213.

¹⁵ Bulletin de la Société de Paris, 1895, **15** : 1125-1126.

mercury in proportion to the amount of protein, is carried down therewith. The precipitation is made with a mercurial salt of known strength and the excess of the mercurial salt in the filtrate is determined by titration. For the details of the manipulation, the paper cited above may be consulted.

518. Separation of Casein from Albumin.—Sebelien prefers magnesium sulfate or sodium chlorid to acetic acid as the best reagent for separating casein from lactalbumin. Of the two saline reagents mentioned, the former is the better. The milk is first diluted with a double volume of the saturated saline solution and then the finely powdered salt added until saturation is secured. The casein is completely thrown out by this treatment, collected on a filter, washed with the saturated saline solution, and the nitrogen therein determined. The difference between the total and casein nitrogen gives the quantity due to the albumin plus the almost negligible quantity due to globulin.¹⁶

519. Van Slyke's Method of Estimating Casein.—The casein may be separated from the other albuminoids in milk by the procedure proposed by Van Slyke.¹⁷ Ten grams of the fresh milk are diluted with 90 cubic centimeters of water and the temperature raised to 40°. The casein is thrown down with a 10 per cent. solution of acetic acid, of which about one and a half cubic centimeters are required. The mixture is well stirred and the precipitate allowed to subside. The whey is decanted onto a filter, the precipitate washed two or three times with cold water, brought finally onto the filter and washed once or twice with cold water. The filter paper and its contents are used for the determination of nitrogen in the usual way. The casein is calculated from the nitrogen found by multiplication by the factor 6.38. Milk may be preserved for this method of determination by adding to it one part of finely powdered mercuric chlorid for each 2,000 parts of the sample. The method is not applicable to curdled milk.

520. Theory of Precipitation.—Most authorities now agree in supposing that the state of semi-solution in which the casein is

¹⁶ *Zeitschrift für Physiologische Chemie*, 1889, **13** : 160.

¹⁷ *Journal of the American Chemical Society*, 1893, **15** : 644.

held in milk is secured by the presence of mineral matters in the milk, in some intimate combination with the casein. Among these bodies lime is of the most importance. The action of the dilute acid is chiefly on these mineral bodies, releasing them from combination with the casein, which, being insoluble in the milk serum, is precipitated.

521. Factors for Calculation.—Most analysts still use the common protein factor, 6.25, in calculating the quantity of proteins from the nitrogen determined by analysis. For casein many different factors have been proposed. According to Makris the factor for human milk varies from 6.83 to 7.04.¹⁸ Munk gives 6.34 for human and 6.37 for cow milk.¹⁹ Sebelien adopts the latter factor, and Hammersten nearly the same, *viz.*, 6.39. The weight of authority, at the present time, favors a factor considerably above 6.25 for calculating the casein and, in fact, the total proteins of milk from the weight of nitrogen obtained. The factor 6.38 has therefore been adopted by the Official Association of Agricultural Chemists.

522. Bechamp's Method of Preparing Pure Casein.—The casein in about one liter of milk is precipitated by adding gradually about three grams of glacial acetic acid diluted with water. The addition of the acid is arrested at the moment when litmus paper shows a slightly acid reaction. The precipitate thus produced, containing all the casein, the milk globules and the microzymes, is separated by filtration, being washed by decantation before collecting it on the filter. On the filter it is washed with distilled water and the fat removed by shaking with ether. The residue is suspended in water, dissolved in the least residue (microzymes, globules) separated by filtration and the pure casein thrown out of the filtrate by the addition of acetic acid. The washing with distilled water, solution in ammonium carbonate, filtration and reprecipitation are repeated three or four times in order to obtain the casein entirely free of other substances. Casein thus

¹⁸ Handbuch der Physiologisch- und Pathologisch-Chemischen Analyse 1909 [8] : 489.

¹⁹ Zeitschrift für Biologie, 1896, 33 n.f. 15 : 64.

prepared is burned to a carbon free ash with difficulty and contains but little over one-tenth per cent. of mineral matter.²⁰

523. Separation of Casein with Carbon Dioxid.—The supersaturation of the lime compounds of casein with carbon dioxid diminishes the solvent action of the lime and thus helps to throw out the protein matter. For this reason Hoppe-Seyler recommends the use of carbon dioxid to promote the precipitation of the casein.²¹ The milk is diluted with about 20 volumes of water and treated, drop by drop, with very dilute acetic acid as long as a precipitate is formed. A stream of pure carbon dioxid is conducted through the mixture for half an hour, and it is allowed to remain at rest for 12 hours, when the casein will have all gone down and the supernatant liquid will be clear. Albumins and peptones are not thrown out by this treatment.

The method of precipitation is advantageously modified by saturating the diluted milk with carbon dioxid before adding the acetic acid, less of the latter being required when used in the order just noted.²²

524. Separation of Albumin.—In the filtrate from the casein precipitate the albumin may be separated by heating to 80°. It may also be precipitated by tannic acid, in which case it contains a little globulin. It may also be thrown out by saturation with ammonium or zinc sulfates. The latter reagent is to be preferred when the nitrogen is to be determined in the precipitate. The quantities of albumin and globulin, especially the latter, present in milk are small compared with its content of casein.

525. Separation of Globulin.—The presence of globulin in milk is demonstrated by Sebelien in the following manner:²³ The milk is saturated with finely powdered common salt and the precipitate produced is separated by filtration. This filtrate in turn is saturated with magnesium sulfate. The precipitate produced

²⁰ Bulletin de la Société Chimique de Paris, 3^e Série, 1894, 11 : 152.

²¹ Hoppe-Seyler, Handbuch der Physiologisch- und Pathologisch-Chemischen Analyse [8] , 1909 : 723.

²² Zeitschrift für Nahrungsmittel-Untersuchung, 1896, 10 : 104.

²³ Zeitschrift für Physiologische Chemie, 1885, 9 : 445.

by this reagent is collected on a filter, dissolved in water and precipitated by saturation with sodium chlorid. This process is repeated several times. The final precipitate is proved to be globulin by the following reactions: When a solution of it is dialyzed the protein body separates as a flocculent precipitate, which is easily dissolved in a weak solution of common salt. The clear solution thus obtained becomes turbid on adding water, and more so after the addition of a little acetic acid. A neutral solution of the body is also completely precipitated by saturation with sodium chlorid. These reactions serve to identify the body as a globulin and not an albumin. All the globulin in milk is not obtained by the process, since a part of it is separated with the casein in the first precipitation.

526. Other Precipitants of Milk Proteins.—Many other reagents besides those mentioned have been used for precipitating milk proteins, wholly or in part. Among these may be mentioned the dilute mineral acids, lactic acid, rennet, mercuric iodid in acetic acid, phosphotungstic acid, acid mercuric nitrate, lead acetate and many others.

It has been shown by the author that many of these precipitants do not remove all the nitrogen but that among others the mercury salts are effective.²⁴ When nitrogen is to be subsequently determined the acid mercuric nitrate cannot be employed.

527. Precipitation by Dialysis.—Since the casein is supposed to be held in solution by the action of salts it is probable that it may be precipitated by removing these salts by dialysis.

528. Carbohydrates in Milk.—The methods of determining lactose in milk, both by the copper reduction and optical processes, have been fully set forth in foregoing paragraphs 118, 148, 184, 186. In general, the optical method by double dilution is to be preferred as practically exact and capable of application with the minimum consumption of time.²⁵ For normal milks a single polarization is entirely sufficient, making an arbitrary correction for the volume occupied by the precipitated proteins and

²⁴ American Chemical Journal, 1884-1885, 6 : 289.

²⁵ Journal of the American Chemical Society, 1896, 18 : 428.

fat. This correction is conveniently placed at six and a half per cent. of the volume of milk employed.

The polarimetric estimation of lactose in human milk is likely to give erroneous results by reason of the existence in the serum of polarizing bodies not precipitable by the reagents commonly employed for the removal of proteins.²⁶ The same statement may be made in respect of ass and mare milk. The use of aceto-picric acid for removing disturbing bodies, as proposed by Thibault²⁷ does not insure results free from error. With the milks above mentioned, it is safer to rely on the data obtained by the alkalin copper reagents.

529. Dextrinoid Body in Milk.—In treating the precipitate, produced in milk by copper sulfate, with alcohol and ether for the purpose of removing the fat, Ritthausen isolated a dextrin like body quite different from lactose in its properties.²⁸ The alcohol ether extract evaporated to dryness leaves a mass not wholly soluble in ether, and therefore not composed of fat. This residue extracted with ether, presents flocky particles, soluble in water and mostly precipitated therefrom by alcohol. This body has a slight reducing effect on alkalin copper salts and produces a gray color with bismuth nitrate. The quantity of this material is so minute as to lead Ritthausen to observe that it does not sensibly affect the fat determinations when not separated. It is not clearly demonstrated that it is a dextrinoid body and the analyst need not fear that the optical determination of milk sugar will be sensibly affected thereby.

Raumer and Späth assume that certain discrepancies, observed by them in the data obtained for lactose by the copper and optical methods, are due to the presence of this dextrinoid body, but no positive proof thereof is adduced.²⁹

530. Amyloid Bodies in Milk.—Herz has observed in milk a body having some of the characteristics of starch.³⁰ Observed

²⁶ *Journal de Pharmacie et de Chimie*, 6^e Série, 1896, 4 : 65.

²⁷ *Contribution à l'Étude des Lactoses*, Thèse pour le diplôme supérieure de Pharmacie, Paris, 1892.

²⁸ *Journal für Praktische Chemie*, 1877, 83, Neue Folge, 15 : 348.

²⁹ *Zeitschrift für angewandte Chemie*, 1896, : 72.

³⁰ *Chemisches Central-Blatt*, 1892, 2 : 1028.

by the microscope, these particles have some of the characteristics of the starch grains of vegetables, with a diameter of from 10 to 35 micromillimeters. They are colored blue by iodine. When boiled with water, however, these particles differ from starch in not forming a paste. The particles are most abundant in the turbid layer found immediately beneath the ether fat solution in the areometric process of Soxhlet.

The amyloid particles may be collected from cheese and butter by boiling with water, when they settle and can be observed on the sediment after freeing of fat by ether.

Some of the statements regarding the adulteration of dairy products with starch may have been made erroneously by reason of the natural occurrence of these particles.

As in the case of the dextrin like body mentioned above this starchy substance, if it really exist, occurs in too minute a quantity to influence the results of any of the analytical methods heretofore described.

In connection with the supposed presence of an amyloid body in milk, it should be remembered that certain decomposition nitrogenous bodies give practically the same reactions as are noted above. Among these may be mentioned chitin, which occurs very extensively in the animal world. The proof of the existence of dextrinoid and amyloid bodies in milk rests on evidence which should be thoroughly revised before being undoubtedly accepted.

531. Official Methods for the Analysis of Dairy Products.—The official methods of analysis adopted for use in the United States in the examination of dairy products are in such common use as to warrant their insertion in full. Some of the more important principles of analysis have already been illustrated by citing portions of these methods but even at the risk of duplicating to some extent the description of the analytical work it is advisable to produce them in convenient form.

1. Total Solids.—*Official.*—(a) *Method I.*—Heat from three to five grams of milk at the temperature of boiling water until it ceases to lose weight, using a tared flat dish of not less than five

cubic centimeters diameter. If desired from 15 to 20 grams of pure dry sand may be previously placed in the dish. Cool in a desiccator and weigh rapidly to avoid absorption of hygroscopic moisture.

(b) *Method II.—(Babcock Asbestos Method.)*—Provide a hollow cylinder of perforated sheet metal, 60 millimeters long and 20 millimeters in diameter, closed five millimeters from one end by a disk of the same material. The perforations should be about 0.7 millimeter in diameter and about 0.7 millimeter apart. Fill loosely with from 1.5 to 2.5 grams of freshly ignited, wooly asbestos, free from fine and brittle material, cool in a desiccator, and weigh. Introduce a weighed quantity of milk (between three and five grams) and dry at the temperature of boiling water to constant weight.

2. *Ash.—Official.*—Weigh about 20 grams of milk in a weighed dish, add six cubic centimeters of nitric acid, evaporate to dryness, and ignite at a temperature just below redness until the ash is free from carbon.

3. *Total Nitrogen.—Official.*—Place about five grams of milk in a Kjeldahl digestion flask and proceed, without evaporation, as described under "I. Fertilizer Methods" for the determination of nitrogen. Multiply the percentage of nitrogen by 6.38 to obtain nitrogen compounds.

4. *Casein and Albumin in Cow's Milk.—(a) Casein.—Official.*—The determination should be made when the milk is fresh, or nearly so. When it is not practicable to make this determination within 24 hours, add one part of formaldehyd to 2,500 parts of milk, and keep in a cool place. Place about 10 grams of milk in a beaker with about 90 cubic centimeters of water at 40° to 42° C., and add at once 1.5 cubic centimeters of a 10 per cent. acetic acid solution. Stir with a glass rod and let stand from three to five minutes longer. Then decant on filter, wash two or three times with cold water by decantation, and transfer precipitate completely to filter. Wash once or twice on filter. The filtrate should be clear, or very nearly so. If it be not clear when it first runs through, it can generally be made so by two or three repeated filtrations, after which the washing of the precipitate can be completed. Determine nitrogen in the washed precipitate and filter paper by the Kjeldahl or Gunning method. To calculate the equivalent amount of casein from the nitrogen multiply by 6.38.

In working with milk which has been kept with preservatives, the acetic acid should be added in small proportions, a few drops at a time, with stirring, and the addition continued until the liquid above the precipitate becomes clear, or very nearly so.

(b) *Albumin*.—*Provisional*.—Exactly neutralize with caustic alkali the filtrate obtained in the preceding operation (a), add 0.3 cubic centimeter of a 10 per cent. solution of acetic acid and heat the liquid to the temperature of boiling water until the albumin is completely precipitated, collect the precipitate on a filter, wash, and determine the nitrogen therein. Nitrogen multiplied by 6.38 equals albumin.

(c) *Casein*.—*Optional Official*.—To 10 cubic centimeters of milk add 50 cubic centimeters of distilled water at 40° C., then add two cubic centimeters of alum solution saturated at 40° or higher. Allow precipitate to settle, transfer to a filter, and wash. Treat the precipitate and filter paper by the Kjeldahl or Gunning method.

(d) *Albumin*.—*Optional Provisional*.—To the filtrate obtained from the casein determination (c) add 0.3 cubic centimeter of a 10 per cent. acetic-acid solution, boil the liquid until the albumin is completely precipitated and proceed as in the provisional method for albumin (b).

5. *Milk Sugar. (Lactose)*.—(a) *Optical Method*.—*Official*.—(1) *Preparation of Reagents*.—(a) *Acid mercuric nitrate*.—Dissolve mercury in double its weight of nitric acid, specific gravity 1.42, and dilute with an equal volume of water. One cubic centimeter of this reagent is sufficient for the quantities of milk mentioned below. Larger quantities may be used without affecting the results of polarization.

(b) *Mercuric iodid with acetic acid*.—Mix 33.2 grams of potassium iodid, 13.5 grams of mercuric chlorid, 20 cubic centimeters of glacial acetic acid, and 640 cubic centimeters of water.

(2) *Determination*.—The milk should be at a constant temperature, and its specific gravity determined with a delicate hydrometer. When greater accuracy is required, a pycnometer is used.

The quantities of the milk measured for polarization vary with the specific gravity of the milk as well as with the polariscope used. The quantity to be measured in any case will be found in the following table:

DETERMINATION OF VOLUME OF MILK SAMPLE.

Specific gravity.	Volume of milk to be used.	
	For polariscopes of which the sucrose normal weight is 16.19 grams.	For polariscopes of which the sucrose normal weight is 26.048 grams.
	cc	cc
1.024	60.0	64.4
1.026	59.9	64.3
1.028	59.8	64.15
1.030	59.7	64.0
1.032	59.6	63.9
1.034	59.5	63.8
1.035	59.35	63.7

Place the quantity of milk indicated in the table in a flask graduated at 102.4 cubic centimeters for a Laurent or 102.6 cubic centimeters for a Ventzke polariscope, (Molir flask). Add one cubic centimeter of mercuric nitrate solution or 30 cubic centimeters of mercuric iodid solution (an excess of these reagents does no harm), fill to the mark, agitate, filter through a dry filter, and polarize. It is not necessary to heat before polarizing. In case a 200 millimeter tube is used, divide the polariscope reading by three when the sucrose normal weight for the instrument is 16.19 grams, or by two when the normal weight for the instrument is 26.048. When a 400 millimeter tube is used, these divisors become six and four, respectively. For the calculation of the above table the specific rotary power of lactose is taken as 52.53° , and the corresponding number for sucrose as 66.5° . The lactose normal weight to read 100° on the sugar scale for Laurent instruments is 20.496 grams, and for Ventzke instruments, 32.975 grams. In case metric flasks are used the weights here mentioned must be reduced to 16.160 and 26.000 grams, respectively for pure sucrose, from which the respective weights of lactose are readily calculated.

(b) *Gravimetric Method.*—Official.—(1) *Preparation of the Milk Solution.*—Dilute 25 cubic centimeters of the milk with 400 cubic centimeters of water and add 10 cubic centimeters of a solution of copper sulfate of the strength given for Soxhlet's modification of Fehling's solution; add about 7.5 cubic centimeters of a solution of potassium hydroxid of such strength that one volume of it is just sufficient to completely precipitate the copper as hydroxid from one volume of the solution of copper sulfate. Instead of a solution of potassium hydroxid of this strength 8.8 cubic centimeters of a half-normal solution of sodium hydroxid may be used. After the addition of the alkali solution

the mixture must still have an acid reaction and contain copper in solution. Fill the flask to the 500 cubic centimeter mark, mix, and filter through a dry filter.

(2). *Determination*.—Place about five grams of milk in a Kjeldhal digestion flask and proceed, without evaporation, as described under "I. Fertilizer Methods" for the determination of nitrogen. Multiply the percentage of nitrogen by 6.38 to obtain nitrogen compounds.

6. *Fat*.—(a) *Babcock Asbestos Method*.—*Official*.—Extract the residue from the determination of water by the Babcock asbestos method with anhydrous ether until all the fat is removed, evaporate the ether, dry the fat at the temperature of boiling water, and weigh. The fat may also be determined by difference, drying the extracted cylinders at the temperature of boiling water.

(b) *Papar Coil Method*.—*Official*.—Make coils of thick filter paper, cut into strips 6.25 by 6.25 cubic centimeters, and thoroughly extract with ether and alcohol, or correct the weight of the extract by a constant obtained for the paper. From a weighing bottle or weighing pipette transfer about five grams of milk to the coil, care being taken to keep the end of the coil held in the fingers dry. Dry the coil, dry end down, on a piece of glass at the temperature of boiling water for one hour; or, better, in hydrogen at the temperature of boiling water; transfer to an extraction apparatus, and extract with absolute ether or petroleum ether boiling at about 45°; dry the extracted fat and weigh.

(c) *Volumetric Methods*.—*Official*.—Babcock's or Gerber's centrifugal methods may be used. Following is the Babcock centrifugal method:

(1) *Apparatus*.—(a) *Babcock milk-test bottles*, graduated to 10 per cent.

(b) *A centrifuge* with sockets for from two to 32 bottles, according to the number of tests to be made, and capable of being run at a speed of from 600 to 1,200 revolutions per minute, according to the diameter of the machine. If many tests are made steam turbine testers or electrical testers will be found convenient.

(c) *Pipettes*, 17.6 cubic centimeters.

(d) *Graduates*, 17.5 cubic centimeters, or a Swedish acid bottle delivering that amount, for measuring sulfuric acid.

(2) *Determination*.—Pipette 17.6 cubic centimeters of the carefully mixed sample into a test bottle and add 17.5 cubic centimeters of commercial sulfuric acid (specific gravity, 1.82-1.83).

Mix, and when the curd is dissolved whirl the test bottles in the centrifugal for four minutes at the required speed for the machine used. Add boiling water, filling to the neck of the bottles, and whirl for one minute; again add boiling water so as to bring the fat within the scale on the neck of the bottles, and after whirling for one minute more read the length of the fat column, care being taken to make the readings at a temperature between 130° and 150° F. when the fat is wholly liquid. The readings give the per cent. of fat in the milk direct.

For details as to the manipulation of the Babcock test and its application in the analysis of dairy products other than milk standard authorities may be consulted.*

7. *Detection of Added Water.*—*Zeiss Immersion Refractometer Method.*—*Provisional.*—To 100 cubic centimeters of milk at a temperature of about 20° add two cubic centimeters of 25 per cent. acetic acid (specific gravity 1.035) in a beaker, and heat the beaker, covered with a watch glass, in a water bath for 20 minutes at a temperature of 70°. Place the beaker in ice water for ten minutes and separate the curd from the serum by filtering through a 12.5 centimeter folded filter. Transfer about 35 cubic centimeters of the serum to one of the beakers that accompanies the control-temperature bath used in connection with the Zeiss immersion refractometer, and take the refractometer reading at exactly 20°, using a thermometer graduated to tenths of a degree. A reading below 39 indicates added water; between 39 and 40 the sample is suspicious.

8. *Detection of Gelatin.*—*Provisional.*—Prepare an acid solution of mercuric nitrate by dissolving mercury in twice its weight of nitric acid of 1.42 specific gravity, and diluting this solution to 25 times its bulk with water. To 10 cubic centimeters of the milk or cream to be examined, add an equal volume of the acid mercuric nitrate solution, shake the mixture, add 20 cubic centimeters of water, shake again, allow to stand five minutes, and filter. If much gelatin is present the filtrate will be opalescent and can not be obtained quite clear. To a portion of the filtrate contained in a test tube, add an equal volume of a saturated aqueous solution of picric acid. A yellow precipitate will be produced in presence of any considerable amount of gelatin, while smaller amounts will be indicated by cloudiness. In the absence of gelatin the filtrate obtained will remain perfectly clear.

* Farrington and Woll, *Testing Milk and Its Products*, 18th Edition, 1908, and Van Slyke, *Modern Methods of Milk Testing*, 1906.

11. *Detection of Benzoic Acid.—Provisional.*—Add five cubic centimeters of dilute hydrochloric acid to 50 cubic centimeters of the milk in a flask and shake to curdle. Then add 150 cubic centimeters of ether, cork the flask and shake well. Break up the emulsion which forms by aid of a centrifuge, or if the latter is not available extract the curdled milk by gently shaking with successive portions of ether, avoiding the formation of an emulsion. Transfer the ether extract (evaporated to small volume if large in bulk) to a separatory funnel and separate the benzoic acid from the fat by shaking out with dilute ammonium hydroxid, which takes out the former as ammonium benzoate. Evaporate the ammoniacal solution in a dish over the water bath till all free ammonia has disappeared, but before dryness is reached add a few drops of ferric chlorid reagent. The characteristic flesh-colored precipitate indicates benzoic acid. Care should be taken not to add the ferric chlorid until all the ammonia has been driven off, otherwise a precipitate of ferric hydrate is formed.

12. *Detection of Salicylic Acid.—Provisional.*—Proceed exactly as directed for benzoic acid in the preceding section. On applying the ferric chlorid to the solution after evaporation of the ammonia the well-known violet color indicates salicylic acid.

13. *Detection of Foreign Color.—(a) Leach's Method.—Provisional.*—Warm about 150 cubic centimeters of milk in a casserole over the flame and add about five cubic centimeters of acetic acid, after which slowly continue the heating nearly to the boiling point while stirring. Gather the curd, when possible, into one mass by the stirring rod, and pour off the whey. If the curd breaks up into small flecks separate from the whey by straining through a sieve of colander. Press the curd free from adhering liquid, transfer to a small flask, and macerate for several hours (preferably overnight) in about 50 cubic centimeters of ether, the flask being tightly corked and shaken at intervals.

(1) *Detection of Annatto (in the ether extract).*—Decant the ether extract as obtained above into an evaporating dish, place on the water bath, and evaporate the ether. Make the fatty residue alkaline with sodium hydroxid, and pour upon a very small wet filter while still warm. After the solution has passed through, wash the fat from the filter with a stream of water and dry the paper. If, after drying, the paper is colored orange, the presence of annatto is indicated. Confirm by applying a drop of stannous chlorid solution, which, in presence of annatto, produces a characteristic pink on the orange-colored paper.

(2) *Detection of Anilin Orange (in the curd).*—The curd of an uncolored milk is perfectly white after complete extraction with ether, as is also that of a milk colored with annatto.

If the extracted fat-free curd is distinctly dyed an orange or yellowish color, anilin orange is indicated. To confirm the presence of this color, treat a lump of the fat-free curd in a test tube with a little strong hydrochloric acid. If the curd immediately turns pink, the presence of anilin orange is assured.

(3) *Detection of Caramel (in the curd).*—If the fat-free curd is colored a dull brown, caramel is to be suspected. Shake a lump of the curd, as in (2), with strong hydrochloric acid in a test tube and heat gently. In the presence of caramel the acid solution will gradually turn a deep blue, as will also the white, fat-free curd of an uncolored milk, while the curd itself does not change color. It is only when this blue coloration of the acid occurs in connection with a brown colored curd, which itself does not change color, that caramel is to be suspected, as distinguished from the pink coloration produced at once under similar conditions by anilin orange.

(b) *Lythgoe's Test for Anilin Orange.*—*Provisional.*—Treat about 10 cubic centimeters of the milk with an equal volume of hydrochloric acid (specific gravity 1.20) in a porcelain casserole and give the dish a slightly rotary motion. If an appreciable amount of anilin orange is present, a pink color will at once be imparted to the curd particles as they separate.

Cream.—Most of the methods for milk analysis may be applied to cream, properly diluted. For accurate work the dilution should in most cases be made after the cream has been weighed (not measured) for the determination, since cream tends to separate quickly from water. In the Babcock centrifugal method the cream should always be weighed into the test bottle before diluting.

Condensed Milk.—*Provisional.*—1. *Preparation of Sample.*—Mix thoroughly by transferring the contents of the can to a large evaporating dish and stirring it with a pestle until homogeneous. Weigh 40 grams of the mixed sample in a 100 cubic centimeter flask, or transfer thereto by washing, and make up to the mark with water.

2. *Total Solids.*—Dilute a measured portion of the above 40 per cent. solution with an equal amount of water, use five cubic centimeters of the diluted mixture, corresponding to 1 gram of the condensed milk, and proceed as directed under Method I, page 622.

3. *Ash*.—Ignite the total solids at very low redness, cool, and weigh.

4. *Proteins*.—Determine nitrogen according to the Kjeldahl or Gunning method in five cubic centimeters of the 40 per cent. solution and multiply by 6.38.

5. *Lactose*.—Dilute five cubic centimeters of the 40 per cent. solution to about 40 cubic centimeters and add 0.6 cubic centimeters of Fehling's copper solution. Nearly neutralize with sodium hydroxid, make up to 100 cubic centimeters, filter through a dry filter, and determine lactose as described under 5, page 624.

6. *Sucrose*.—Determine by difference, deducting the milk solids (lactose+proteins+fat+ash) from the total solids.

7. *Fat or Ether Extract*.—Place 15 cubic centimeters of the 40 per cent. solution in a Babcock test bottle. Fill the bottle nearly to the neck with water, add 4 cubic centimeters of Fehling's copper solution, shake thoroughly and rapidly, separating the precipitated proteins and fat by means of a centrifuge,³¹ or the precipitate may be allowed to settle, which it does more quickly in the cold. Withdraw the supernatant liquid by means of a small-stemmed pipette with a wisp of wet absorbent cotton twisted over the bottom to serve as a filter. Wipe off the cotton into the bottle on withdrawing the pipette. Give the precipitated proteins and fat two additional washings by shaking with water, separating the precipitate, and removing the washings with the pipette. If the precipitate is caked hard after centrifuging, use a stiff platinum wire as a stirrer. Finally, add water to an approximate volume of 17.5 cubic centimeters and 17.5 cubic centimeters of sulfuric acid, and continue the test as in the Babcock process of milk testing, multiplying the reading by three for the percentage of fat in the sample.

ANALYSIS OF BUTTER.

532. General Principles.—The general analysis of butter fat is conducted in accordance with the methods described in the part of this volume devoted to the examination of fats and oils. The methods of sampling, drying, filtering, and of determining physical and chemical properties, are there developed in sufficient detail to guide the analyst in all operations of a general nature.

³¹ While the steam-driven centrifuge may be used, it is better to centrifuge in the cold, since the heat of the steam-driven machine cakes the precipitate so that it is harder to wash.

There remain for consideration here only the special processes practiced in butter analysis and which are not applied to fats in general. These processes naturally relate to the study of those properties of a distinctive nature, by means of which butter is differentiated from other fats for which it may be mistaken or with which it may be adulterated. These special studies, therefore, are directed chiefly to the consideration of the peculiar physical properties of butter fat, to its content of volatile acids and to its characteristic forms of crystallization as observed with the aid of the microscope. For dietetic, economic and legal reasons, it is highly important that the analyst be able to distinguish a pure butter from any substitute therefor.

533. Appearance of Melted Butter.—Fresh, pure butter, when slowly melted, shows after a short time the butter fat completely separated, of a delicate yellow color and quite transparent. Old samples of butter do not give a fat layer of equal transparency. Oleomargarin, or any artificial butter when similarly treated, gives a fat layer opalescent or opaque. By means of this simple test an easy separation of pure from adulterated butter may be effected. In mixtures, the degree of turbidity shown by the separated fats may be regarded as a rough index of the amount of adulteration. In conducting the work, the samples of butter, in convenient quantities according to the size of the containing vessel, are placed in beakers and warmed slowly at a temperature not exceeding 50°. After a lapse of half an hour the observations are made.

If one part of the melted butter be shaken with two volumes of warm water (40°) and set aside for five minutes the fat is still found as an emulsion, while oleomargarin, similarly treated, shows the fat mostly separated. This process has some merit, but must not be too highly valued.⁸²

534. Microscopic Examination of Butter.—The microscope is helpful in judging the purity of butter and the admixture of foreign fats, it not in too small quantity to be of any commercial

⁸² Zeitschrift für Nahrungsmittel-Untersuchung, 1896, 10 : 86.

importance, can easily be detected by this means.³³ The methods of preparing butter fat in a crystalline state are the same as those described in paragraphs 331-333. The crystals of butter fat differ greatly in appearance with the different methods of preparation. When butter is melted, filtered, heated to the boiling point of water and slowly cooled, it forms spheroidal crystalline masses as seen by the microscope, which present a well defined cross with polarized light. This cross is not peculiar to butter fat, but is developed therein with greater distinctiveness than in other fats of animal origin.

Pure, fresh, unmelted butter, when viewed with polarized light through a plate of selenite, presents a field of vision of uniform tint, varying with the relative positions of the nicols. When foreign fats, previously melted, as in rendering, are mixed with the butter the crystallization they undergo disturbs this uniformity of tint and the field of vision appears particolored. Old, rancid or melted butter may give rise to the same or similar phenomena under like conditions of examination. The microscope thus becomes a most valuable instrument for sorting butters and in distinguishing them in a preliminary way from oleo-margarin.

535. Judgment of Suspected Butter or Lard by Refractive Power.—In discriminating between pure and adulterated butters by the aid of the butyrefractometer (326), the absolute reading of the instrument is of less importance than the difference which is detected between the highest permissible numbers, for and degree of temperature, and the actual reading obtained at that temperature. These differences, within certain limits, do not perceptibly vary with the temperature, and heretofore they have been determined with the aid of a table, and in this respect the observations have been made the more laborious.

Wollny has rendered these tables unnecessary by constructing a thermometer in which the mercury column does not indicate degrees of temperature, but the highest permissible number for butter or lard at the temperature of observation. The scale of

³³ Bulletin 13, Part 1, Division of Chemistry, 1887 : 29 et seq.

the instrument is so adjusted as to include temperatures of from 30° to 40° , which renders it suited to the examination of butter and lard. The oleothermometer is shown in Fig. 105.

The side of the scale *B* is for butter and that marked *S* for lard. The sample of fat is placed in the prisms in the usual

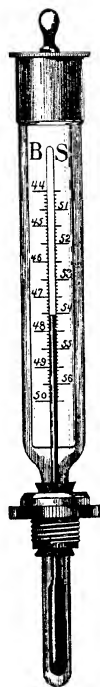


Fig. 105.—Thermometer for Butyrefractometer.

manner. When the mercury in the thermometer is at rest, the scale of the instrument is read. In the case of a butter, if the reading of the scale give a higher number than that indicated by the thermometer, the sample is pronounced suspicious and the degree of suspicion is proportional to the difference of the two readings.

536. Official Methods of Butter Analysis.³⁴—*Butter and its Substitutes.*—1. *Preparation of Sample.*—If large quantities of butter are to be sampled, use a butter trier or sampler. Completely melt the portions thus drawn, 100 to 500 grams, in a closed vessel at as low a temperature as possible. When melted, cool the whole, and at the same time shake the mass violently until it is homogeneous and sufficiently solidified to prevent the separation of the water and fat. Then pour a portion into the vessel from which it is to be weighed for analysis. The sample should completely or nearly fill the vessel and should be kept in a cold place until analyzed.

2. *Moisture.*—Place 1.5 to 2.5 grams in a dish with a flat bottom having a surface of at least 20 square centimeters and dry at the temperature of boiling water until it ceases to lose weight, each drying to be for only one hour. The use of clean dry sand or asbestos is admissible and is necessary if a dish with a round bottom be employed.

3. *Casein, Ash, and Chlorin.*—Cover the crucible containing the residue from the fat determination by the indirect method (see 4 (a)) and heat gently at first, gradually raising the temperature to just below redness. The cover may then be removed and the heat continued until the contents of the crucible are white. The loss in weight represents casein, and the residue in the crucible, mineral matter. In this mineral matter, dissolved in water slightly acidulated with nitric acid, determine chlorin, either gravimetrically or volumetrically.

4. *Ether Extract.*—(a) *Indirect Method.*—Dissolve in the dish with absolute ether or petroleum ether the dry butter obtained in the water determination in which no absorbent was used, transfer to a weighed gooch with the aid of a wash bottle filled with the solvent and wash until free from fat. Dry the crucible and contents at the temperature of boiling water until the weight is constant and calculate the fat.

(b) *Direct Method.*—From the dry butter obtained in determining the water, either with or without the use of an absorbent,

³⁴ Bureau of Chemistry, Bulletin 107 (revised), 1912 : 123-126.

extract the fat with anhydrous alcohol-free ether, receiving the solution in a weighed flask. Evaporate the ether and dry the extract at the temperature of boiling water until it ceases to lose weight, the dryings not to exceed one hour each in duration.

5. *Salt*.—Weigh in a counterpoised beaker from five to 10 grams of butter, using portions of about 1 gram from different parts of the sample. Add about 20 cubic centimeters of hot water and after the butter is melted transfer the whole to a separatory funnel. Insert the stopper and shake for a few moments. Let stand until the fat has all collected on the top of the water, then draw off the latter into a flask, being careful to let none of the fat globules pass. Again add hot water to the beaker and repeat the extraction from 10 to 15 times, using each time from 10 to 20 cubic centimeters of water. The washings will contain all but a mere trace of the sodium chlorid originally present in the butter. Determine its amount in the whole or an aliquot of the liquid by the volumetric silver-nitrate method, with potassium chromate as indicator.

6. *Examination of Fat*.—(a) *Preparation of Sample*.—Melt the butter and keep it in a dry place at about 60° for two or three hours, until the water and curd have entirely separated. Pour off the clear supernatant fat and filter through a dry filter paper in a hot-water funnel containing boiling water, or in an oven at about 60°. Should the filtered liquid fat not be perfectly clear it must be filtered again.

(b) *Methods of Examination of Butter Fat*.—

7. *Qualitative Tests of Butter*.—(a) *Microscopic Examination*.—Place a small portion of the fresh unmelted sample, taken from the inside of the mass, on a slide, add a drop of pure sweet oil, cover with gentle pressure, and examine with a one-half to one-eighth inch objective for crystals of lard, etc. Examine the same specimen with polarized light and a selenite plate without the use of oil. Pure fresh butter will neither show crystals nor a particolored field with selenite. Other fats melted and cooled and mixed with butter will usually present crystals and variegated colors with the selenite plate.

For further microscopic study dissolve from three to four cubic centimeters of the fat in 15 cubic centimeters of ether in a test tube. Close the tube with a loose plug of cotton wool and allow to stand from 12 to 24 hours at 20° to 25°. When crystals form at the bottom of the tube, they are removed with a pipette, glass rod, or tube, placed on a slide, covered, and examined. The crystals formed by later deposits may be examined in a similar way.

(b) *Special Tests for Renovated (Process) Butter and Oleo.*—

(1) *Foam Test.*—Heat two or three grams of the sample, either in a spoon or dish, over a free flame. True butter will foam abundantly, whereas process butter will bump and sputter, like hot grease, without foaming. Oleo behaves like process butter, but chemical tests will determine whether the sample is oleo-margarine or butter.

(2) *Appearance of the Melted Fat.*—Melt from 50 to 100 grams of butter or process butter at 50°. The curd from butter will settle, leaving a clear supernatant fat. On the other hand, the supernatant fat in the case of process butter does not assume that clear appearance, but remains more or less turbid.

(3) *Microscopic Examination.*—Place a bit of the butter or process butter on a glass slide, cover it and press it into a thin film with cover glass. Examine immediately with a polarizing microscope magnifying from 100 to 140 diameters. When a selenite plate is placed between the slide and the lower nicol a normal butter will give a uniformly blue colored field, showing the absence of fat crystals.

(c) *Detection of Boric Acid.*—Melt about 25 grams of the sample on the water bath, pour off the fat from the aqueous solution that settles, slightly acidify the aqueous solution with hydrochloric acid, and test in the usual manner with turmeric paper for boric acid.

(d) *Detection of Annatto and Saffron.*—(1) *Cornwall's Method.*—*Provisional.*—Dissolve five grams of the fat in 50 cubic centimeters of ether in a wide tube and shake the solution vigorously with 12 to 15 cubic centimeters of a very dilute solu-

tion of potassium hydroxid, which must still be alkaline after it separates from the ether solution. Allow to stand a few hours, then draw off the aqueous layer, evaporate to dryness, and test with sulfuric acid which in the presence of annatto gives first a blue or violet blue, changing quickly to green and finally to brown.

Saffron, which would be extracted at the same time, acts differently when treated with sulfuric acid, not giving the green coloration.

The aqueous solutions, if not clear enough to use, must not be filtered, as the filter paper will take up large amounts of the color, but can be shaken up again with fresh portions of ether. Carbon disulfid may also be used as a solvent. Uncolored butters treated in this way give only a very slight trace of coloring matter.

(2) *Massachusetts Board of Health Method for Annatto.*—Treat two or three grams of the melted and filtered fat (freed from salt and water) with warm dilute sodium hydroxid, and after stirring pour the mixture while warm upon a wet filter, using to advantage a hot funnel. If annatto is present the filter will absorb the color so that when the fat is washed off by a gentle stream of water the paper will be dyed straw color. It is well to pass the warm, alkaline filtrate two or three times through the fat on the filter to insure removal of the color. If, after drying the filter, the color turns pink on application of a drop of stannous chlorid solution the presence of annatto is assured.

(3) *Geisler's Method for Azo Colors.*—Spread a few drops of the clarified fat upon a porcelain surface and add a pinch of Fuller's earth. In the presence of various azo dyes a pink to violet red coloration will be produced in a few minutes. Some varieties of the Fuller's earth react much more readily than others with azo colors.

(4) *Low's Method for Azo Colors.*—Melt a small amount of the fat in a test tube, add an equal volume of the mixture of one part of concentrated sulfuric acid and four parts of glacial acetic acid and heat nearly to the boiling point, the liquids being

thoroughly mixed by shaking. Then set aside and after the acid solution has settled it will be colored wine red in the presence of azo color, while with pure butter fat comparatively no color will be produced.

(5) *Acid and Alkali Tests*.—Pour into each of two test tubes about two grams of the filtered fat dissolved in ether. Into one of the test tubes pour one or two cubic centimeters of hydrochloric acid and into the other about the same volume of dilute potassium hydroxid solution. Shake the tubes well and allow to stand. In the presence of azo dye the test tube to which the acid has been added will show a pink to wine-red coloration, while the potash solution in the other tube will show no color. If, on the other hand, annatto or other vegetable color has been used the potash solution will be colored yellow, while no color will be apparent in the acid solution.

537. Volatile or Soluble Acids.—The distinguishing feature of butter, from a chemical point of view, is found in its content of volatile or soluble fat acids. Among the volatile acids are reckoned those which are carried over in a current of steam at a temperature only slightly higher than that of boiling water. As soluble acids are regarded those which pass without great difficulty into solution in hot water. These two classes are composed essentially of the same acids. Of these butyric is the most important, followed by caproic, caprylic and capric acids. Small quantities or rather traces of acetic, lauric, myristic and arachidic acids are also sometimes found in butter. Palmitic, stearic and oleic acids also occur in large quantities. The above named acids, in combination with glycerol, form the butter fat.

538. Relative Proportion of Ingredients.—The composition of butter fat is given differently by different authorities.³⁵ A typical dry butter fat may be regarded as having the following composition:

	Per cent.
Butyrin.....	7.00
Caproin, Caprylin and Caprin.....	2.30
Olein.....	37.70
Palmitin, stearin, etc.	53.00

³⁵ Benedikt and Lewkowitsch *Chemical analysis of Oils, Fats and Waxes*, 1898, [2] : 606.

Pure butter fat consists principally of the above glycerids, some coloring principles, varying in quantity and composition with the food of the animal, and a trace of lecithin, cholesterol, phytosterol and a lipochrome.

539. Estimation of Volatile or Soluble Acids.—The volatile or soluble acids in butter fat are estimated by the methods already described (389-391). In practice preference is given to the method of determining volatile acids, based on the principle that under standard conditions practically all the acids of this nature are secured in a certain volume of the distillate. This assumption is not strictly true, but the method offers a convenient and reliable manner of obtaining results which, if not absolute, are at least comparative.

The quantity of acid distilled is determined by titration with tenth normal alkali and for convenience the data are expressed in terms of the volume of the alkali consumed. Five grams of normal butter fat will give a distillate, under the conditions given, requiring about 28 cubic centimeters of tenth normal alkali for complete saturation. This is known as the Reichert-Meissl number. Occasionally this number may rise to 32 or may sink to 25. Cases have been reported where it fell below the latter number, but such samples cannot be regarded as normal butter.

The determination of the Reichert-Meissl number is the most important of the chemical processes applied to butter fat analysis.

540. Saponification Value and Reichert Number.—It may often be convenient to make the same sample of butter fat serve both for the determination of the saponification value and of the Reichert number. For this purpose it is convenient to use exactly five grams of the dry filtered fat. The saponification may be accomplished either under pressure or by attaching a reflux condenser to the flask as suggested by Bremer.³⁶ When the saponification, which is accomplished with alcoholic potash lye containing about 1.25 grams of potassium hydrate in each 10 cubic centimeters of 70 per cent. alcohol, is finished, and the contents of the flask are cooled, the residual alkali is titrated with a set sulfuric

³⁶ *Forschungs-Berichte über Lebensmittel*, 1895, 2 : 424.

Chemiker-Zeitung Repertorium, 1896, 20 : 15.

acid solution, using phenolphthalein as indicator. When the color has almost disappeared, an additional quantity of the indicator is added and the titration continued until the liquid is of an amber tint. A sample of the alkali, treated as above, is titrated at the same time and from the two sets of data obtained, the saponification number is calculated as indicated in paragraph 384.

A few drops of the alcoholic lye are added to the contents of the flask and the alcohol removed by evaporation. The residual soap and potassium sulfate are dissolved in 100 cubic centimeters of recently boiled water, some pieces of pumice added, and the volatile acids removed by distillation in the usual way after adding an excess of sulfuric acid. It is important to conduct blank distillations in the same form of apparatus to determine the magnitude of any corrections to be made. The size of the distilling flask and the form of apparatus to prevent mechanical projection of sulfuric acid into the distillate should be the same in all cases.

541. Modification of the Reichert-Meissl Method.—Kreis has proposed the use of strong sulfuric acid for saponifying the fats, the saponification and distillation being accomplished in one operation. A source of error of some inconvenience in this method is due to the development of sulfurous acid by the reducing action of the organic matter on the oil of vitriol. Pinette proposes to avoid this difficulty by adding, before the distillation is begun, sufficient potassium permanganate to produce a permanent red coloration. By this means the sulfurous acid is completely oxidized and its transfer to the standard alkali during distillation entirely prevented. The same result is accomplished by Micko by the use of potassium bichromate. The details of the manipulation are as follows:³⁷

About five grams of the fused fat (butter or oleomargarine) are placed in a flask of approximately 300 cubic centimeters capacity. After cooling, there are added 10 cubic centimeters of sulfuric acid containing three grams of water to each 97 grams of the strongest acid.

The fat and acid are well mixed by a gentle rotatory motion of the flask and placed in a water-bath at a temperature of 35°

³⁷ *Revue Internationale des Falsifications*, Mai 1893, 6 : 157.

(*circa*) for 15 minutes. At the end of this time the flask is removed from the bath and 125 cubic centimeters of water added, little by little, keeping the contents cool. Next are added four cubic centimeters of a four per cent. solution of potassium bichromate. The contents of the flask are vigorously shaken and, after five minutes, a solution of ferrous sulfate is added gradually from a burette until the reaction with a drop of potassium ferrocyanid shows a slight excess of the iron salt. The volume of the liquor in the flask is then increased to 150 cubic centimeters by the addition of water and 110 cubic centimeters distilled. After mixing and filtering through a dry filter, the acid in 100 cubic centimeters is determined by standard tenth normal barium hydroxid solution and the number thus obtained increased by one-tenth representing the total acid obtained.

542. Elimination of Sulfurous Acid.—Prager and Stern³⁸ propose to eliminate the sulfurous acid by a stream of air, succeeded by one of carbon dioxid, and proceed as follows: Five grams of the butter fat are brought into a liter flask, 10 cubic centimeters of strong sulfuric acid are added and the flask is kept for 10 minutes at 30°-32° with constant agitation. When the liquid is cold, air is bubbled through it until the odor of sulfurous acid has disappeared. One hundred cubic centimeters of water are added, with precautions against rise of temperature, and carbon dioxid is bubbled through for 10 minutes. This is then displaced by a stream of air for another 10 minutes, the delivery tube is washed into the flask with 50 cubic centimeters of water and the distillation is effected. The following results are quoted:

Cubic centimeters of tenth normal alkali required by five grams of butter fat:

	Reichert-Meissl.	Prager-Stern.
Sample <i>a</i>	29.86	29.60
" <i>b</i>	30.23	29.65
" <i>c</i>	28.34	27.76
" <i>d</i>	28.20	28.10

The authors do not comment on the possibility of loss of acids other than sulfurous in the stream of air, but they admit that further investigation is requisite to render the suggestion of Kreis serviceable.

³⁸ Chemiker-Zeitung, 1899, 17 : 468.

543. Errors Due to Poor Glass.—The easy solubility of the glass holding the reagents is the cause of some of the difficulties attending the determination of the saponification value. The separated silica tends to carry down, mechanically, a part of the alkali. This is shown by the fact that after the color has been discharged by titration with acid and the flask set aside a reappearance of the red color is noticed, after a time, beginning at the bottom of the flask.³⁹ In order to avoid difficulties of this nature, either cold saponification should be practiced or the digestion vessels used for moist combustion in sulfuric acid be employed.

Errors may also be easily introduced by the use of uncalibrated burettes and from the employment of varying quantities of the phenolphthalein solution.

544. Estimation of the Molecular Weight of Butter and Butter Substitutes.—Garelli and Carcano have proposed a method for discriminating between butter and its substitutes by the cryoscopic determination of molecular weights.

The molecular weights of stearin, palmitin and olein are 890, 806 and 884, and of butyrin, caproin and caprylin 303, 386 and 470 respectively. Pure butter, therefore, has a lower mean molecular weight than margarin.

The method and apparatus of Beckmann are used in the determination, 15 grams of benzol being employed as a solvent.

The constant for the molecular depression of the benzol is found to be 53.

The molecular weight obtained with samples of pure butter varied from 696 to 716, and for oleomargarine from 780 to 883.

The figures obtained with mixtures of 20, 25, 33 and 50 per cent. of margarin with butter were 761, 720, 728 and 749 respectively. The method can be relied upon to classify samples as follows:

1. Pure butter.
2. Butter containing margarin.
3. Suspicious butter.⁴⁰

³⁹ Zeitschrift für angewandte Chemie, 1896, : 177.

⁴⁰ Zeitschrift für Nahrungsmittel-Untersuchung, 1894, 8 : 219.

Le Stazioni Sperimentali Agrarie Statione, 1893, 25 : 77.

545. Substitutes and Adulterants of Butter.—In this country, butter is never adulterated with cocoa or sesame oil, as is sometimes the case in other lands. The common substitute for butter here is oleomargarine, and the most common butter adulterant, neutral lard. The methods of analyses, by means of which these bodies can be identified, have already been sufficiently described. By the use of certain digestive ferments and other bodies, butter may be made to hold an excessive quantity of casein, sugar and water in the form of a somewhat permanent emulsion.⁴¹ This form of adulteration is revealed at once on melting the sample.

546. Furfural Reaction with Sesame Oil.—Olive oil and sometimes butter are mixed with the cheaper body, sesame oil. The latter is detected with certainty from the red coloration it gives when mixed with furfural and hydrochloric acid. Instead of furfural, some body yielding it when subjected to the action of hydrochloric acid, *viz.*, sucrose or a pentose sugar, may be used. It has been found by Wauters, however, that an alcoholic solution of two grams of furfuraldehyd in 100 cubic centimeters of alcohol is the best reagent. One-tenth of a cubic centimeter of this reagent is used for each test.⁴²

The test is made as follows: The quantity of the furfuraldehyd solution mentioned above is mixed with 10 cubic centimeters of hydrochloric acid, and there are added, without mixing, an equal volume of the suspected oil. On standing, a red coloration is produced at the zone of separation of the two liquids. If the oil be sesame, the coloration is produced instantly. As little as one per cent. of sesame in a mixed oil will show the color in two minutes. The manipulation is also varied by shaking together the reagents and the melted butter. Turmeric, which is sometimes used in coloring butter, also gives the rose red color when treated with hydrochloric acid, but turmeric supplies its own furfuraldehyd. It is easy to distinguish therefore the coloration due to sesame oil, which is developed only when furfuraldehyd is present, from that due to the turmeric, which is produced without the aid of the special reagent.

⁴¹ Farmers' Bulletin 12, U. S. Department of Agriculture, 1893 : 4-5.

⁴² Bulletin de l'Association Belge des Chimistes, 1895-96, 9 : 279.

547. Butter Colors.—Where cows are deprived of green food and root crops, such as carrots, and kept on a poorly balanced ration, the butter made from their milk may be almost colorless. To remedy this defect it is quite a common practice to color the product artificially. Almost the sole coloring matters used in this country are anatto and yellow coal tar dyes.⁴³ Other coloring matters which are occasionally employed are turmeric, saffron, marigold leaves, yellow wood (*Chlorophora tinctoria*), and carrot juice. Chrome yellow (lead chromate) and dinitrocresol are not used in this country.

The use of small quantities of anatto, turmeric or saffron is unobjectionable from a sanitary, but is not to be excused from an ethical point of view.

EXAMINATION OF CHEESE.

548. Composition of Cheese.—Pure cheese is made from whole milk by precipitating the casein with rennet. The precipitated casein carries down also the fat of the milk and a little lactose and whey remain incorporated with the cheesy mass. The ingredients of cheese are therefore those of the whole milk less the greater part of the whey, *id est*, milk sugar, lactalbumin, globulin, soluble mineral matters and water. In the conversion of the crude precipitate noted above into the cheese of commerce, it is subjected to a ripening process which is chiefly conditioned by bacterial action. It is not possible here to enter into a discussion of methods of isolating and identifying the bacteria which promote or retard the ripening process.⁴⁴ The most important changes during ripening take place in the protein matter, which is so altered as to become more palatable and probably more digestible as a result of the bacterial and enzymic activity.

The percentage composition of the principal cheeses of commerce are shown in the following table:⁴⁵

⁴³ Bulletin 13, Part 1, Division of Chemistry, 1887 : 26.

⁴⁴ Russell; Outlines of Dairy Bacteriology, 1902 [5] : 160.

⁴⁵ Woll; Dairy Calendar, 1895 : 223.

	Water, Per cent.	Casein, Per cent.	Fat Per cent.	Milk sugar, Per cent.	Ash, Per ct.
Cheddar.....	34.38	26.38	32.71	2.95	3.58
Cheshire	32.59	31.51	26.06	4.53	4.31
Stilton	30.35	28.85	35.39	1.59	3.83
Brie	50.35	17.18	25.12	1.94	5.42
Neuchâtel	44.47	14.60	33.70	4.24	2.99
Roquefort	31.20	27.63	33.16	2.00	6.01
Edam.....	36.28	24.06	30.26	4.60	4.90
Swiss.....	35.80	24.44	37.40	...	2.36
Whole milk, mean of 143 analysis	38.60	25.35	30.25	2.03	4.07

It is evident that the composition of the cheese will vary with the milk from which it is made and the manipulation to which it is subjected.* A good American green cheese made from milk of the composition noted below will have the composition which is appended.⁴⁶

TABLE SHOWING MEAN COMPOSITION OF MILK AND CHEESE
MADE THEREFROM.

	Milk.	Cheese.
Per cent. water.....	87.38	36.70
“ fat	3.73	34.18
“ proteins	3.13	23.44
“ milk sugar, ash, etc.....	5.76	5.68

From the above it is seen that in whole milk cheese the ratio of fat to casein is 1.46:1, and to solids not fat 1.17:1. This is a point of some importance in judging the purity of a cheese. When the whole milk of a mixed herd is used the percentage of fat in a cheese will always be considerably higher than that of casein.

549. Manipulation of the Milk.—When sweet milk is received at the cheese factory, a starter of sour milk is added to it in order to hasten its ripening. When it is thought that the proper degree of acidity has been secured, it is subjected to a rennet test. In this test 160 cubic centimeters of the milk are heated to 30° and mixed with five cubic centimeters of the rennet solution made by diluting five cubic centimeters of the rennet of commerce with 50 cubic centimeters of water. The number of seconds required for the milk to curdle is noted. The observa-

⁴⁶ Van Slyke; Bulletin 82, New Series, New York Agricultural Experiment Station, 1894: 654-655.

tion is facilitated by distributing throughout the milk a few fine fragments of charcoal. The contents of the vessel are given a circular motion and, at the moment of setting, the movement of the black particles is suddenly arrested. The quantity of rennet required is determined by the nature of the cheese which it is desired to make. For a cheese to be rapidly cured, enough rennet should be added to produce coagulation in from 15 to 20 minutes, and when slow curing is practiced in from 30 to 45 minutes. When the mass is solid so that it can be cut with a knife, the temperature is raised to 37° , and it is tested on a hot iron until it forms threads an eighth of an inch in length. This test is made by applying an iron heated nearly to redness to the curd. When the curd is in proper condition threads from a few millimeters to two centimeters in length are formed, when the iron is withdrawn. The longer threads indicate, but only to a limited extent, a higher degree of acidity.⁴⁷ This test is usually made about two and one-half hours from the time of coagulation. The whey is then drawn off through a strainer and the curd is placed on racks with linen bottoms in order that the residual whey may escape. In from 15 to 20 minutes it can be cut into blocks eight or 10 inches square and turned over. This is repeated several times in order to facilitate the escape of the whey. When the curd assumes a stringy condition, it is run through a mill and cut into small bits and is ready for salting, being cooled to 27° before the salt is added. From two to three pounds of salt are used for each 100 pounds of curd. The curd is then placed in the molds and pressed into the desired form. The cheeses thus prepared are placed on shelves in the ripening room and the rinds greased. They should be turned and rubbed every day during the ripening, which takes place at a temperature of from 15° to 18° .⁴⁸

550. Official Methods of Analysis.—The methods of cheese analysis recommended by the Association of Official Agricultural

⁴⁷ Babcock ; Wisconsin Agricultural Experiment Station, Twelfth Annual Report, 1896 : 133

⁴⁸ Woll ; Dairy Calendar, 1895 : 220.

Chemists are provisional and are not binding on its members. They are as follows:⁴⁹

1. *Selection and Preparation of Sample.*—When the cheese can be cut take a narrow wedge-shaped segment reaching from the outer edge to the center of the cheese. Cut this into strips and pass through a sausage grinding machine three times. When the cheese can not be cut take the sample with a cheese trier. If only one plug can be obtained take it perpendicular to the surface of the cheese at a point one-third of the distance from the edge to the center and extending either entirely or half way through it. When possible draw three plugs—one from the center, one from a point near the outer edge, and one from a point half way between the other two. For inspection purposes reject the rind, but for investigations requiring the absolute amount of fat in the cheese include the rind in the sample. It is preferable to grind the plugs in a sausage machine, but when this is not done they are cut very fine and carefully mixed.

2. *Moisture.*—Place from two to five grams of cheese in a weighed platinum or porcelain dish which contains a small quantity of porous material, such as ignited asbestos or sand, to absorb the fat which may run out. Heat in a water oven for 10 hours and weigh; the loss in weight is considered as moisture. Or, if preferred, the dish may be placed in a desiccator over concentrated sulfuric acid and dried to constant weight. In some cases this may require as much as two months. The acid should be renewed when the cheese has become nearly dry.

Modification of Official Method.—A modification of the official method has the advantage of greatly facilitating drying.

Into a flat bottomed platinum dish about 6.5 centimeters in diameter, place about two grams of finely ground ignited asbestos, pressing it down well over the bottom, and put in the dish a thick glass rod, cut off square at one end, to serve as a pestle. Ignite and weigh the apparatus and add about five grams of the cheese sample (prepared as usual) and with the glass rod rub and press the cheese and asbestos together most intimately until

⁴⁹ Bulletin 107 (revised), Bureau of Chemistry, 1912 : 126.

all cheese particles have disappeared and the mass is homogeneous; finally loosen the mass up into as fluffy a condition as possible and place in the drying oven. Dry until loss of weight ceases, each drying being for only one hour or an hour and a half. Usually two or three dryings suffice.⁵⁰

3. *Ash*.—The dry residue from the moisture determination may be used for the ash. If the cheese be rich in fat, the asbestos will be saturated therewith. Ignite cautiously to avoid spurting, removing the lamp while the fat is burning off. When the flame has died out, the burning may be completed in a muffle at low redness. When desired, the salt may be determined in the ash in the manner specified under butter analysis.

4. *Nitrogen*.—Determine nitrogen by the Kjeldahl or Gunning method, using about two grams of cheese, and multiply the percentage of nitrogen by 6.38 to obtain the nitrogen compounds.

5. *Fat*.—(a) *Gravimetric Method*.—Cover the perforations in the bottom of the extraction tube with dry asbestos felt, and on this place a mixture containing equal parts of anhydrous copper sulfate and pure, dry sand to the depth of about five centimeters, packing loosely. Cover the upper surface of this material with a film of asbestos. Place on this two to five grams of the sample and extract with anhydrous ether for five hours in a continuous extraction apparatus. Remove the cheese and grind it to a fine powder with pure sand in a mortar, replace the mixed cheese and sand in the extraction tube, washing the mortar with ether and adding the washings to the tube, and continue the extraction for at least 10 hours.

(b) *Babcock Centrifugal Method*.—Weigh about six grams of cheese in a tared dish. Add 10 cubic centimeters of boiling water and stir with a rod until the cheese softens and an even emulsion is formed, preferably adding a few drops of strong ammonium hydroxid, and keep the beaker in hot water until the emulsion is nearly completed and the mass free from lumps.

If the sample is a whole milk cheese a Babcock cream bottle is employed.

⁵⁰ Bureau of Chemistry, Bulletin 116, 1908 : 59.

The contents of the beaker, after cooling, are transferred to the test bottle as follows: Add to the beaker about half of the 17.6 cubic centimeters of sulfuric acid usually employed in this test, stir with a rod, and pour carefully into the bottle, using the remainder of the acid in two portions for washing out the beaker. Finally proceed as in the Babcock test for milk. Multiply the fat reading by 18 and divide by the weight of the sample to obtain the per cent of fat.

6. *Acidity*.—To 10 grams of finely divided cheese add water at the temperature of 40° until the volume equals 105 cubic centimeters; agitate vigorously and filter. Titrate 25 cubic centimeter portions of the filtrate with standard sodium hydroxid, preferably tenth-normal, using phenolphthalein as indicator. Express amount of acid as lactic.

7. *Separation of Fat for Examination*.—(a) *Method I—Alkaline Extraction Method*.—Cut about 300 grams of the cheese into fragments the size of a pea. Treat with 700 cubic centimeters of potassium hydroxid (50 grams per liter) at 20° in a large wide-necked flask, and promote the solution of casein by vigorous shaking. In from five to 10 minutes the casein will be dissolved and the fat will come to the surface in lumps. Collect the lumps of fat into as large a mass as possible by gently shaking. Pour cold water into the flask until the fat is driven up into the neck and remove it by means of a spoon. Wash the fat thus obtained with as little water as will remove the residue of the lye which it may contain. Experience has shown that the fat is not perceptibly attacked by the lye in this treatment. By this method the fat is practically all separated in a short time and is then easily prepared for chemical analysis by filtering and drying as directed in the official method.

(b) *Method II—Acid Extraction Method*.—Grind the cheese by passing it through a meat-cutting machine. Transfer it to a large flask and pour warm water upon it using one cubic centimeter for every gram of cheese. Shake thoroughly and add sulfuric acid (specific gravity 1.82 to 1.825) slowly and in small quantities, shaking after each addition of acid. The total

amount of acid used should be the same as the amount of water. Remove the fat, which separates after standing a few minutes, by means of a separatory funnel, wash it free from acid, filter, and dry.

551. Process of Mueller.—The process of Mueller,⁵¹ as modified by Kruger, is conducted as follows: About ten grams of a good average sample of cheese are rubbed in a porcelain mortar with a mixture of three parts of alcohol and one part of ether. After the mixed liquids have been in contact with the cheese five or 10 minutes they are poured upon a weighed filter of from 15 to 16 centimeters diameter, and this process is repeated from one to three times, after which the contents of the mortar are brought upon the filter. The filtrate is received in a weighed flask, the alcohol ether driven off by evaporation and the residue dried. Since it is difficult to get all the particles of cheese free from the mortar, it is advisable to perform the above process in a weighed dish which can afterwards be washed thoroughly with ether and alcohol and dried and the amount of matter remaining thereon accounted for. The residue remaining in the flask after drying is treated several times with pure warm ether, and the residue also remaining upon the filter mentioned above is completely extracted with ether. The dried residue obtained in this way from the filter plus the residue in the flask which received the filtrate, plus the amount left upon the dish in which the cheese was originally rubbed up, constitute the total dry matter of the cheese freed of fat. All the material soluble in ether should be collected together, dried and weighed as fat.

By this process the cheesy mass is converted into a fine powder which can be easily and completely freed from fat by ether, and can be dried without becoming a gummy or horny mass.

For the estimation of the nitrogen, about three grams of the well grated cheese are used and the nitrogen determined by moist combustion with sulfuric acid.⁵²

⁵¹ Landwirthschaftliches Jahrbuch, 1872, 1 : 68.

⁵² Principles and Practice of Agricultural Analysis, 1908, 2 [2] : 346.

For the estimation of ash, about five grams are carbonized, extracted with water, and the ash determined as described below.⁵³

Char from two to three grams of the substance and burn to whiteness at the lowest possible red heat. If a white ash cannot be obtained in this manner, exhaust the charred mass with water, collect the insoluble residue on a filter, burn, add this ash to the residue from the evaporation of the aqueous extract and heat the whole to a low redness till the ash is white.

552. Schmidt-Bondzynski Method.—This process is a combination, with slight modifications, of two methods, namely, the Werner-Schmidt Method⁵⁴ and the Bondzynski Method.⁵⁵ In the former method ethyl ether only is used for extracting the fat; in the latter petroleum ether only is used.

Place about one gram of the prepared sample in a small beaker, add nine cubic centimeters of water and one of concentrated ammonia, heat on a steam bath (stirring with a glass rod) until an emulsion is formed. Neutralize the ammonia with concentrated hydrochloric acid and add 10 cubic centimeters of hydrochloric acid, place on a wire gauze over a flame, boil for five minutes, cool, transfer to a Rohrig tube and extract with mixed solvents (sulfuric and petroleum ethers), as in the Roesse-Gottlieb method for milks.

553. Filled Cheese.—The skim milk coming from the separators is unfortunately too often used for cheese making. The abstracted fat is sometimes replaced with a cheaper one, usually lard. These spurious cheeses are found in nearly every market and are generally sold as genuine. The purchasers only discover the fraud when the cheese is consumed. Many of the States have forbidden by statute the manufacture and sale of this fraudulent article. Imported cheeses may also be regarded with suspicion, inasmuch as the method of preparing filled cheese is well known and extensively practiced abroad. A more determination of the percentage of fat in the sample is not an index of

⁵³ Division of Chemistry, Bulletin 46, 1895 : 24.

⁵⁴ Leach; Food Adulteration and Analysis : 160.

⁵⁵ Analysis, 1904, 28 : 190.

the purity of the cheese. It is necessary to extract the fat by one of the methods already described and, after drying and filtering, to submit the suspected fat to a microscopic and chemical examination. A low content of volatile fat acid and the occurrence of crystalline forms foreign to butter will furnish the data for a competent judgment.

When the Reichert-Meissl number falls below 25 the sample may be regarded with suspicion. The detection of the characteristic crystals of lard or tallow is reliable corroborating evidence.

It is stated by Kühn⁵⁶ that the margarine factory of Mohr, at Bahrenfeld-Altona, has made for many years a perfect emulsion of fat with skim milk. This product has been much used in the manufacture of filled cheese.

554. Separation of the Nitrogenous Bodies in Cheese.—The general methods of separation already described for protein bodies (419-430) are also applicable to the different nitrogenous bodies present in cheese, representing the residue of these bodies as originally occurring in the milk, and also the products which are formed therefrom during the period of ripening. For practical dietary and analytical purposes, these bodies may be considered in three groups

(a) The useless (from a nutrient point of view) nitrogenous bodies, including ammonia, nitric acid, the phenylamido-propionic acids, tyrosin, leucin and other amid bodies.

(b) The proteins and peptones, products of fermentation soluble in boiling water.

(c) The caseins and albuminates, insoluble in boiling water.

The group of bodies under (a), according to Stutzer, may be separated from the groups (b) and (c) by means of phosphotungstic acid. For this purpose a portion of an intimate mixture of fine sand and cheese (100 cheese, 400 sand) corresponding to five grams of cheese, is shaken for 15 minutes with 150 cubic centimeters of water. After remaining at rest for another 15 minutes 100 cubic centimeters of dilute sulfuric acid (one acid.

⁵⁶ Chemiker Zeitung, 1895, 19 : 554.

three water) are added, followed by treatment with the phosphotungstic acid as long as any precipitate is produced. The mixture is thrown on a filter and the insoluble matters washed with dilute sulfuric acid until the filtrate amounts to half a liter. Of this quantity an aliquot part (200 cubic centimeters) is used for the determination of nitrogen. From the quantity of nitrogen found, that representing the ammonia, as determined in a separate portion, is deducted and the remainder represents the nitrogen present in the cheese as amids.⁵⁷

Proteoses and Peptones.—Proteoses and peptones are determined in cheese by the following method:⁵⁸ A quantity of the sand mixture already described, corresponding to five grams of the cheese, is treated with about 100 cubic centimeters of water, heated to boiling, and the clear liquid above the sand poured into a flask of half a liter capacity. The extraction is continued with successive portions of water in like manner until the volume of the extract is nearly half a liter. When cold, the volume of the extract is completed to half a liter with water, the liquor filtered, 200 cubic centimeters of the filtrate treated with an equal volume of dilute sulfuric acid (one to three) and phosphotungstic acid added until no further precipitate takes place. The nitrogen is determined in the precipitate after filtration and washing with dilute sulfuric acid.

Casein and Albuminates.—The quantity of casein and albuminates in cheese is calculated by subtracting from the total nitrogen that corresponding to ammonia, amids, that in the indigestible residue and that corresponding to the proteose and peptone. In three samples of cheese, *viz.*, camembert, swiss, and gervais, Stutzer found the nitrogen, determined as above, distributed as follows:⁵⁹

	Camembert.	Swiss.	Gervais.
N as ammonia.....	13.0	3.7	1.6
N as amids.....	38.5	9.0	5.2
N as proteose peptone ...	30.5	8.6	15.5
N indigestible.....	4.0	2.4	8.6
N as casein, albuminates.	14.0	76.3	69.1

⁵⁷ Zeitschrift für analytische Chemie, 1896, **35** : 497.

⁵⁸ Zeitschrift für analytische Chemie, 1896, **35** : 499.

⁵⁹ Zeitschrift für analytische Chemie, 1896, **35** : 502.

Ammoniacal Nitrogen.—The ammoniacal nitrogen is determined by mixing a quantity of the sand-cheese corresponding to five grams of cheese, with 200 cubic centimeters of water, adding an excess of barium carbonate and collecting the ammonia by distillation in the usual way.

Digestible Proteins.—The digestible proteins in cheese are determined by the process of artificial digestion, which will be described in the part of this volume treating of the nutritive value of foods.

These data show the remarkable changes which the proteins undergo where the ripening is carried very far as in the camembert cheese.

555. Koumiss.—Fermented mare milk has long been a favorite beverage in the East, where it is known as koumiss. In Europe and this country cow milk is employed in the manufacture of fermented milk, although it is less rich in lactose than mare milk. The process of manufacture is simple, provided a suitable starter is at hand. A portion of a previous brewing is the most convenient one, the fermentation being promoted by the addition of a little yeast. After the process of fermentation is finished the koumiss is placed in bottles and preserved in a horizontal position in a cellar, where the temperature is not allowed to rise above 12°. Properly fermented milk containing only a small quantity of alcohol is a grateful food in many cases of disordered digestion or in other diseases which cause digestive disorders.

556. Determination of Carbon Dioxid.—The carbon dioxid in koumiss is conveniently estimated by connecting the bottle by means of a champagne tap with a system of absorption bulbs.⁶⁰ The exit tube from the koumiss bottle passes first into an Erlenmeyer, which serves to break and retain any bubbles that pass over. The water is next removed from the gas by means of sulfuric acid. The koumiss bottle is placed in a bath of water which is raised to the boiling point as the evolution of the gas is accomplished. The arrangement of the apparatus is shown in Fig. 106. At the end of the operation any residual carbon dioxid

⁶⁰ Bulletin 13, Part 1, Division of Chemistry, 1887 : 118.

Division of Chemistry, Bulletin 13, Part 2, 1887 : 293.

in the apparatus is removed by aspiration after removing the tap and connecting it with a soda-lime tube to hold back the carbon dioxid in the air. A large balance suited to weighing the koumiss bottle is required for this determination. The carbon

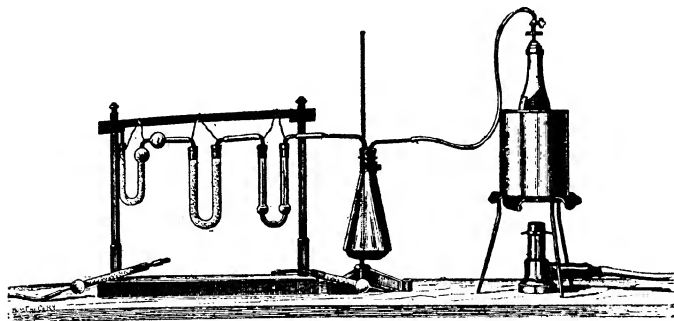


Fig. 106.—Apparatus for Determining Carbon Dioxid in Koumiss.

dioxid may also be determined, but less accurately, by loss of weight in the koumiss bottle after adding the weight of water retained in the apparatus.

557. Acidity.—Although koumiss may contain a trace of acetic acid, its acidity is due chiefly to lactic acid. The preliminary clarification is most easily accomplished by mixing the koumiss with an equal volume of 95 per cent. alcohol, shaking and filtering. The first filtrate will usually be found clear. If not it is refiltered. In an aliquot part of the filtrate the acidity is determined by titration with tenth-normal sodium hydroxid solution, using phenolphthalein as indicator. The necessary corrections for dilution and volume of the precipitated casein are to be made. A linen filter may be used when paper is found too slow. The total acidity is calculated to lactic acid.

558. Alcohol.—Half a liter of koumiss to which 100 cubic centimeters of water have been added, is distilled until the distillate amounts to 500 cubic centimeters.

If the distillate be turbid 100 cubic centimeters of water are added and the distillation repeated. The alcohol is determined by the processes described hereafter.

559. Lactose.—The milk sugar may be determined by any of the methods described, but most conveniently by double dilution and polarization.

560. Fat.—Evaporate 20 grams of the sample to dryness and extract with pure ether or petroleum spirit in the manner already described.

The analysis is more quickly accomplished by the volumetric method of Babcock or Gerber.

561. Water and Ash.—From two to five grams of the koumiss are dried to constant weight in a flat platinum dish over ignited sand, asbestos or pumice stone, and the dried residue incinerated.

562. Composition of Koumiss.—The composition of koumiss varies with the character of the milk used and the extent of the fermentation. Some of the data obtained by analysts are given below:⁶¹

COMPOSITION OF KOUMISS.

Kind of milk.	Water, Per cent.	Sugar, Per cent.	Alcohol, Per cent.	Fat, Per cent.	Protein, Per cent.	Carbon dioxid, Per cent.	Acid- ity, Per cent
Cow.....	89.32	4.38	0.76	2.08	2.56	0.83	0.47
Probably cow skim'd	3.95	1.38	0.88	2.89	0.82
Mare.....	91.87	0.79	2.89	1.19	1.91	...	1.04

From the above it is seen that koumiss is made either from whole or skim milk, and that the percentage of alcohol may vary within large limits, its proportion being inverse to that of the milk sugar.

Koumiss is a beverage which is very palatable, easily digested and one which is not appreciated in this country in proportion to its merits, especially for the use of invalids.

⁶¹ American Chemical Journal, 1886, 8 : 200.

Bulletin 13, Part I, Division of Chemistry, 1887 : 120.

PART SEVENTH

MISCELLANEOUS AGRICULTURAL PRODUCTS.

563. Classification.—In the preceding parts have been set forth the fundamental principles underlying the conduct of agricultural analysis and a résumé of the best practice of the art. The analyst, as a rule, will seldom be required to undertake investigations which are unnoticed in the preceding pages. Cases will arise, however, in which problems are presented which cannot be solved by the rules already elucidated. In respect of the great classes of agricultural bodies, it will be observed that dairy products, sugars and starches have already received special mention. In respect of foods and fodders in general, it is evident that they are chiefly composed of moisture, ash, carbohydrates, oils and protein matters. The methods of identifying, separating and estimating these constituents have been fully set forth. It is not necessary, therefore, to study in this part the analytical processes which are applicable to cereals, cattle foods and other food products, further than is necessary to present in the most important cases a working résumé of principles and methods. There remain, however, certain products of importance which require some special modifications of treatment, and it is to these that the present part will be chiefly devoted. Among these are found tobacco, tea and coffee, fruits, fermented and distilled drinks and certain animal products. It is evident that an enumeration of all agricultural products, with a description of their methods of examination, would be impracticable in the available space and undesirable by reason of the repetition which would be required. In each case the analyst, in possession of the methods described, will be able to adapt the means at his disposal to the desired purpose to better advantage than any rigid directions could possibly secure.

In respect of the analytical methods of determining the nutritive value of foods, they may be divided into chemical and phys-

iological. The chemical methods embrace the thermal and artificial digestion investigations, and the physiological include those which are carried out with the help of the animal organisms. In the latter case the digestive process is checked by the analysis of the foods before ingestion and of the excreta of all kinds during and after digestion.

It is evident that a detailed description of this method should be looked for in works devoted to physiological and biological chemistry.

CEREALS AND CEREAL FOODS.

564. General Analysis.—The cereals are prepared for analysis by grinding until the fragments pass a sieve having circular perforations half a millimeter in diameter. The moisture, ash, ether extract, proteins and carbohydrates are determined by some one of the processes already described in detail. In this country the methods of the Association of Official Agricultural Chemists are generally followed.⁶² For convenience these methods are summarized below.

Moisture.—Dry a convenient quantity of the substance, representing about two grams of dry material, at the temperature of boiling water until it ceases to lose weight (approximately five hours), in a current of dry hydrogen or in vacuo. If the substance be held in a glass vessel the latter should not be in contact with the boiling water.

Ash.—Char a convenient quantity of the substance, representing about two grams of the dry material, and burn until free of carbon at the lowest possible heat. If a carbon-free ash cannot be obtained in this manner, exhaust the charred mass with water, collect the insoluble residue on a filter, burn till the ash is white or nearly so, and then add the filtrate to the ash and evaporate to dryness. Heat the whole to a low redness and weigh.

Crude Fat or Ether Extract.—Wash any of the commercial brands of ether with two or three successive portions of distilled

⁶² Bureau of Chemistry, U. S. Department of Agriculture, Bulletin 107 (revised) 1912: 38-39.

water, add solid sodium or potassium hydroxid, and let stand until most of the water has been abstracted from the ether. Decant into a dry bottle, add carefully cleaned metallic sodium cut into small pieces, and let stand until there is no further evolution of hydrogen gas. The ether thus dehydrated must be kept over metallic sodium, and should be lightly stoppered in order to allow any accumulating hydrogen gas to escape. It may be drawn off with a pipette as required.

Determination.—(1) *Direct Method.*—Extract a convenient quantity of the substance, representing about two grams of the dry material, dried as for the determination of moisture, with anhydrous alcohol-free ether for 16 hours. Dry the extract at the temperature of boiling water for one-half hour, remove from the oven to a desiccator, cool and weigh; continue this alternate drying and weighing at half-hour intervals until a minimum weight of fat is obtained. For most feeds a period of from one to one and one-half hours is required to obtain a minimum weight.

(2) *Indirect Method.*—Determine moisture as above, extract the dried substance for 16 hours as directed under the direct method, dry again and regard the loss of weight as ether extract.

Crude Proteins.—Proceed as in the method of determining nitrogen in the absence of nitrates and multiply the weight of nitrogen obtained by 6.25. This factor is a general one, but should not be rigidly applied. In each instance, according to the nature of the cereal, the appropriate factor, pointed out in paragraph 434 should be used, and the factor 6.25 be applied only in those cases where a special factor is not given. The factors for the common cereals are wheat 5.70, rye 5.62, oats 6.31, maize 6.39, barley 5.82 and flaxseed 5.62.

For separating the protein matters consult paragraphs 419-431.

Amid Nitrogen.—The albuminoid nitrogen is determined as directed in paragraph 338 of Volume II. The difference between this number and that representing the total nitrogen gives the nitrogen as amids.

Fiber and Carbohydrates.—The methods of analysis are described in detail in Part Third of this volume.

564a. Spoiled Maize.—Indian corn is often stored and shipped in a moist state and is prone to develop injurious molds. The consumption of such spoiled products often proves fatal to domesticated animals, and has also, though not with convincing data, been held to be the cause of pellagra. It has been found that moldy, musty or spoiled corn has a greater acidity than the sound product, and the measure of this acidity has been used as a means of discriminating between edible and inedible samples.⁶³

The method of conducting the determination of the acidity of Indian corn, as practiced by the South Carolina station, is as follows: A sample of corn is ground to pass a 20-mesh sieve, 10 grams are placed in a 50 cubic centimeter graduated flask, fitted with a ground glass stopper, the flask is filled to the mark with 85 per cent. alcohol and allowed to stand for 24 hours at room temperature with occasional shaking. The contents are poured upon a dry filter and 25 cubic centimeters filtered into a graduated cylinder. From 100 to 150 cubic centimeters of distilled water are added, with a few drops of phenolphthalein. The titration is conducted with twentieth normal alkali solution, (sodium or potassium hydrate.) The number of cubic centimeters of twentieth normal alkali, multiplied by ten, gives the acidity of a kilogram of corn in terms of cubic centimeters normal alkali.

Experience has shown that the acidity of sound corn, measured in this method, ranges from 13 to 25, that is, it requires from 13 to 25 cubic centimeters of normal alkali to neutralize the extract from a kilogram of sound corn. Whenever the number of cubic centimeters of normal alkali exceeds 30, it is an evidence that the corn is more or less spoiled. Higher acidity indicates a

⁶³ South Carolina Department of Agriculture, Bulletin 29, 1913 : 7.

Alsberg and Black, Bureau of Plant Industry, Department of Agriculture, Bulletin 199, 1911.

Watson, South Carolina Department of Agriculture, Bulletin 23, 1911.

Alsberg and Black, Bureau of Plant Industry, Bulletin 270, 1912.

greater degree of spoilage. One of the acids developed is penicillic.

565. Bread.—In general the same processes are followed in bread analysis as are used with cereals and flours. In addition to the regular analytical processes, breads are to be examined for adulterants, bleaching and coloring matters, and for the purpose of determining the changes which have taken place in their nutrient constituents in the processes of fermentation and cooking.

Temperature of Baking.—The interior of a loaf during the process of baking does not attain the high temperature commonly supposed. This temperature is rarely found to be more than one degree above the boiling point of water. In biscuits and other thin cakes, which become practically dry and which by reason of their thinness are the more readily penetrated by heat, the temperature may go as high as 110° .

Soluble Extract.—The quantity of matters both in flour and bread, soluble in cold water, is determined by extraction in the usual way and drying the extract. Soluble albuminoids, sugars and mineral salts are extracted by this process. When possible, the operation should be conducted both on the bread and the flour from which it is made. In case of flour or other uncooked cereal the flask in which the extraction is made should be packed in ice to prevent, as much as possible, the action of enzymes during the extraction.

Color.—In baker's parlance is found an apparent contradiction of terms, since it speaks of bread with "no color" when the loaf is dark brown, while a white loaf is said to have a high color. An ideal color for the interior of a loaf made of pure wheat flour is a light cream tint, which is more desirable than a pure white.⁶⁴ The texture, odor and flavor of the loaf are also to be considered, but these are properties of more importance to the technical expert than to the analyst. In bread made of the flour of the whole grains the color varies with that of the cereal, being yellow when yellow corn is used and varying from light brown to almost black from wheat and rye.

⁶⁴ Jago. *The Chemistry of Wheat, Flour and Bread*, 1886: 457.

Quantity of Water.—It is not possible to set a rule of limitation in respect of the quantity of water a bread should hold. For full loaves, perhaps 40 per cent. is not too high a maximum, while some authors put it as low as 34 per cent. Some flours are capable of holding more water than others, and the loaf should have just enough water to impart to the slice of bread the requisite degree of softness and the proper texture. Most wheat flour breads will have a content of water ranging from 30 to 40 per cent. In biscuits and other thin cakes the moisture is much less in quantity.

Acidity.—The acidity of both bread and flour is determined by shaking 10 grams of the sample with 200 cubic centimeters of distilled water for 15 minutes, pouring the mass on a filter and titrating an aliquot part of the filtrate with tenth-normal alkali. The acidity is reckoned as lactic acid in the case of breads raised by fermentation.

Nature of Nitrogenous Compounds.—The methods of investigation are described in paragraphs 396-399.

566. Determination of Alum in Bread.—The only alum to be found in bread in this country is that which may be due to the use of alum baking powders. The alum constituent of baking powders is now generally the dehydrated sodium-aluminium sulfate. The presence of alum in bread may be detected by means of logwood. Five grams of fresh logwood chips are digested with 100 cubic centimeters of amyl alcohol. One cubic centimeter of this decoction and the same quantity of a saturated solution of ammonium carbonate are mixed with 10 grams of flour and an equal quantity of water. With pure flour, a slight pink tint is produced. In the presence of alum the color changes to a lavender or blue, which is persistent on heating.

The test may be varied by diluting five cubic centimeters of the reagents mentioned with 90 cubic centimeters of water and pouring the mixture over 10 grams of the crumbled bread. After standing for five minutes, any residual liquid is poured off and the residue, washed once with a little water, is dried in a steam bath, when the blue color is developed if alum be present.⁶⁵

⁶⁵ Jago. *The Chemistry of Wheat, Flour and Bread*, 1886: 465.

567. Simple Tests for Detecting Bleaching in Flour.—During the last few years the practice of bleaching flour, usually with nitrogen oxids has become prevalent, but now appears to have been largely discontinued owing to the enforcement of the food and drugs act. Following are qualitative tests for bleached flour:

I. *Griess-Ilosvay Method.*—Place a heaping teaspoonful (10 grams) of the flour to be examined in a wide-mouthed, glass-stoppered four-ounce bottle. Nearly fill with distilled water, or tap water free from an appreciable amount of nitrates, and add a teaspoonful (four cubic centimeters) of the test solution prepared as directed below, measured with a glass spoon. Cork the bottle and shake vigorously for a few minutes, then allow to settle for from 15 to 20 minutes.

Under the above conditions bleached flour will impart to the liquid a color ranging from a light pink to a deep red, depending on the degree of bleaching. With unbleached flour the liquid is not colored a red tint, provided water free from nitrites is used. Always run, for comparison, a parallel test with a sample of unbleached flour so that allowance can be made for any nitrites in the water.

Test solution:—1. Dissolve 0.5 gram of sulfanilic acid in 150 cubic centimeters of dilute acetic acid (about 20 per cent.). Keep well stoppered.

2. Dissolve 0.2 gram of alpha-naphthylamin hydrochlorid in 20 cubic centimeters of strong acetic acid (glacial), and add 130 cubic centimeters of dilute acetic acid (20 per cent.). Keep well stoppered.

Mix one and two for use. The mixed reagent keeps for several weeks, and possibly much longer.

II. *Gasoline Method.*—Place two heaping teaspoonfuls (20 grams) of flour in a wide-mouthed, glass-stoppered four-ounce bottle, add sufficient gasoline to nearly fill the bottle, shake, and allow to settle. If the flour is unbleached, the gasoline will become distinctly yellow; if bleached, it will remain nearly colorless. Conduct a parallel test on unbleached flour for compari-

son. This process must be conducted in a room where there is no fire.

568. Color Value.—The following method has been suggested by Winton⁶⁶ for determining the color value in flour:

Place 20 grams of the flour in a wide-mouthed, glass stoppered bottle of about 120 cubic centimeters capacity (four ounces) and add 100 cubic centimeters of colorless gasoline. Stopper tightly and shake vigorously for five minutes. After standing 16 hours, shake again for a few seconds until the flour has been loosened from the bottom of the bottle and thoroughly mixed with the gasoline, then filter immediately on a dry 11 centimeter filter into an Erlenmeyer flask, keeping the funnel covered with a watch glass to prevent evaporation. In order to secure a clear filtrate, a certain quantity of the flour should be allowed to pass onto the filter and the first portion of the filtrate passed through a second time. It will be found convenient to fit the filter paper to the funnel by means of water and dry thoroughly either by standing over night in a well ventilated place, or by heating.

Determine the color value of the clear gasoline solution in a Schreiner colorimeter, using for comparison a 0.005 per cent. water solution of potassium chromate. This solution corresponds to a gasoline number of 1.0 and may conveniently be prepared by making 10 cubic centimeters of a 0.5 per cent. solution up to one liter. The colorimeter tube containing the gasoline solution should first be adjusted so as to read 50 millimeters, then the tube containing the standard chromate solution raised or lowered until the shades of yellow in both tubes match. The reading of the chromate solution, divided by the reading of the gasoline solution gives the gasoline color value.

Usually the gasoline solution is a true yellow, but sometimes, especially in the case of clear flour, a slight brownish tint is discernible although this is not sufficient to interfere with the accuracy of the test. Standing for a longer time than prescribed above does not appear to affect the results; in fact, the

⁶⁶ Bureau of Chemistry, Bulletin 122, 1909: 217.

filtration may be dispensed with entirely if the solution is allowed to settle after the second shaking until perfectly clear, which, usually requires at least 24 hours.

If desired, the color value may be determined in Nessler tubes, using for comparison potassium chromate solutions of various dilutions prepared from the 0.5 per cent. stock solution and filling the tubes in all cases to the height of 50 millimeters, or in a Lovibond Tintometer, using a one inch coil and the yellow glass slides. In the latter case, the color is obtained directly, the 0.005 per cent. potassium chromate solution corresponding in color to the 1.0 yellow slides. In order to avoid the complication of two standards, the color value should in all cases be referred to the 0.005 per cent. potassium chromate solution.

If an approximate result is sufficient for the purpose, such may be obtained by carefully pipetting off the clear, supernatant gasoline solution before the second shaking. The results thus obtained are about 10 per cent. lower than by the standard method as above given.

569. Chemical Changes Produced by Baking.—Changes of a chemical nature, produced in bread by baking, are found chiefly in modifications of the starch and proteins. The starch is partly converted into dextrin and the albumins are coagulated. The changes in digestion coefficient are determined by the methods which follow. The fermentations which precede the baking consist essentially in the alcoholic fermentation of the sugar present and the production of lactic acid. There is also a conversion of gluten into soluble proteins induced by some ferment contained in the flour.⁹⁷ A portion of the carbohydrates is also partially caramelized in the crust.

FODDERS, GRASSES AND ENSILAGE.

570. General Principles.—The analyst in examining the fibrous foods of cattle, is expected to determine moisture, ash, fiber and other carbohydrates (nitrogen-free extract) ether extract and albuminoid and amid nitrogen. If a more exhaustive study be

⁹⁷ Carlo Parenti, *Bulletin, chim. pharm.*, 1903, **42** : 353-357.

required, the sugar and starch are separated from the other non-nitrogenous matters, the carbohydrate bodies yielding furfural-dehyd separately determined and the ash subjected to a quantitative analysis. The processes are conducted in harmony with the principles and methods of procedure fully set forth in the preceding pages.

Green fodders and grasses are easily dried and sampled by comminution in the shredder described on page 10, and roots by that shown on page 327. The moisture is determined by drying a small sample of the shredded mass, while the rest of it is dried, first at about 60° and finally at 100°, or a little above, ground to a fine powder and subjected to analysis by methods already described. The food values as obtained by analysis should be compared, when possible, with those secured by natural and artificial digestion.

Ensilage is shredded and analyzed in precisely the same way, but in drying, the content of volatile acids formed during fermentation must be considered. In other words, the loss on drying ensilage at 100°, or slightly above, is due not only to the escape of water but also to the volatilization of the acetic acid, which is one of the final products of fermentation which the mass undergoes in the silo.

571. Organic Acids in Ensilage.—In the examination of ensilage, the organic acids which are present may be determined by the processes described in following paragraphs. The acetic acid, formed chiefly by fermentation, is conveniently determined by the method given for tobacco further on. Lactic acid is detected and estimated by expressing the juice from a sample of ensilage, removing the acetic acid by distillation, repeated once or twice, and treating the filtered residue with zinc carbonate in excess, filtering and determining the zinc lactate in the filtrate. The zinc is determined by the method described for evaporated apples and the lactic acid calculated from the weight of zinc found. Crystallized zinc lactate contains 18.18 per cent. of water and 27.27 per cent. of zinc oxid.⁶⁸

⁶⁸ Richardson, *Journal of the Chemical Society. Transactions.* 1885. 47 : 84.

Other acids which have been found in silage are propionic, butyric and valeric, but acetic acid constitutes about seventy-five per cent. of the total.^{68a} The total acidity of ensilage varies greatly with the age, physical condition and other factors of the environment, and also with the solvent. A part of the acidity is insoluble in water, but soluble in alcohol. Thus the total acidity determined by alcohol extraction, estimated as acetic is greater than when it is determined by water extraction. The acidity of water extraction varies from 1 to 1.5 per cent; by alcohol extraction from 1.75 to 2 per cent. Fineness of grinding is one of the important things to be looked after in securing the maximum degree of acidity.

572. Changes due to Fermentation in the Silo.—Silage differs from green fodder in having less starch and sugar, more acetic and lactic acids and alcohol and a higher proportion of amid to albuminoid nitrogen.⁶⁹ There is also a considerable loss of nitrogenous substances in ensilage, due probably to their conversion into ammonium acetate, which is lost on drying.

573. Alcohol in Ensilage.—The fermentation which takes place in the silo is not wholly of an alcoholic nature, as the development of lactic acid, noted above, clearly indicates. The alcohol which is formed may escape and but small quantities can be detected in the ripened product. So small is this quantity of alcohol that it appears to be useless to try to secure a quantitative estimation of it. Qualitively, it may be detected by collecting it in a distillate, which is neutralized or made slightly alkaline with soda or potash lye and redistilled. The greater part of the alcohol will be found in the first few cubic centimeters, which are made alkaline with potash lye and as much iodine added as can be without giving a red tint to the solution. Any alcohol which is present will soon separate as iodoform.

574. Comparative Values of Fodder and Ensilage.—In judging of the comparative values of green and dry fodders for feeding

^{68a} Connecticut, Storrs Experiment Station, Bulletin 70; Journal of the American Chemical Society, 1912, **34** : 1619; 1913, **35** : 93, 476.

⁶⁹ Richardson, Journal of the Chemical Society. Transactions, 1885, **47** : So et seq.

purposes, it is necessary to secure representative samples in the green, quickly dried and ensilaged condition. It is quite certain that the greater part of the sugar contained in green fodders is lost both by natural curing and by placing in a silo. When well cured by the usual processes there is but little loss of nitrogenous matters, but in the silo this loss is of considerable magnitude, amounting in some instances to as much as 30 per cent.

The ideal way of preparing green fodders in order to preserve the maximum food value efficiently, is to shred them and dry rapidly by artificial heat, or in the sunlight, until they are in a condition which insures freedom from fermentation. In this condition, when placed in bales, under heavy pressure, the food constituents are preserved in the highest available form. The immense sugar content of the stalks of maize and sorghum could be preserved in this way almost indefinitely.

FLESH PRODUCTS.

575. Names of Meats.—The parts of the animal from which the meats are taken have received distinctive names, which serve to designate the parts of the carcass offered for sale. These names are not invariable and naturally are quite different in many markets. In this country there is some degree of uniformity among butchers in naming the meats from different parts. The names in scientific use for the parts of mutton, beef and pork are found in the accompanying illustrations.⁷⁰

576. Sampling.—When possible the whole animal should constitute the sample. The relative weights of blood, intestinal organs, hide, hoofs, horns, bones and edible flesh are determined as accurately as possible. The general method of preparing samples of animal products is given in paragraph 5 (b).

The method of sampling employed by Atwater and Woods is essentially that just noted.⁷¹ The sample, as received at the laboratory, is weighed, the flesh (edible portion) is then sepa-

⁷⁰ Office of Experiment Stations, Bulletin 28, 1896.

⁷¹ Office of Experiment Stations, U. S. Dept. of Agri., Bulletin 29, 1896: 8.

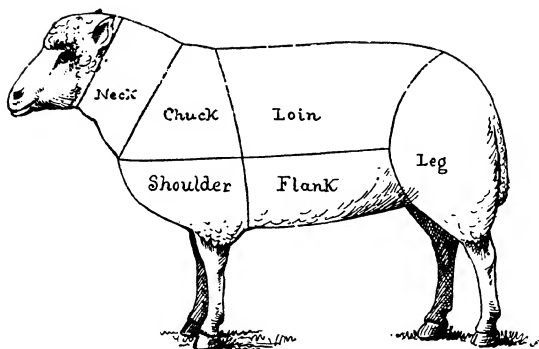


Fig. 107.

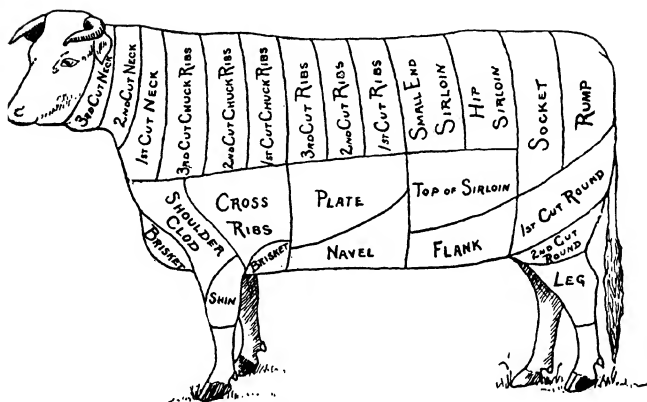


Fig. 108.

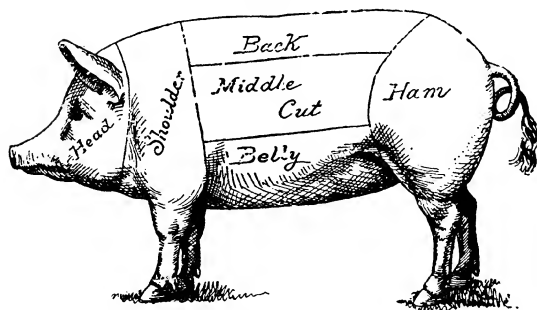


Fig. 109.

rated from the refuse (skin, bones, etc.) and both portions weighed. There is always a slight loss in the separation, evidently due to evaporation and to small fragments of the tissues that adhere to the hands and to the implements used in preparing the sample. The perfect separation of the flesh from the other tissues is difficult, but the loss resulting from this is small. In sampling the material for analysis, it is finely chopped, either in a tray or in a sausage cutter, and in each case is well mixed. Atwater and Woods direct that the sample be dried as described under "water and water-free substance" below, and that the determinations be made on the air-dried sample.

According to the methods of the Association of Official Agricultural Chemists the determinations are made on the fresh undried sample which is prepared as follows:—

Separate the sample as completely as possible from the bones and pass it rapidly and repeatedly through a sausage mill until thorough mixture and complete comminution are obtained. The sample must be kept on ice to prevent decomposition, and all of the determinations should be begun as soon as practicable after the sample is prepared. Dry that portion of the sample which is not needed for analysis, extract with gasoline which boils below 60° C., allow the gasoline to evaporate spontaneously, and expel the last traces by heating for a short time on the steam bath. Neither the meat nor the separated fat should be heated longer than necessary, owing to the tendency of the latter to decompose.⁷²

577. Methods of Analysis.—The general methods for the analyses of food products are applicable to meats and animal products in general. In the separation of the nitrogenous constituents the methods described in paragraphs 398-399 are followed. It is not safe to estimate as proteins the total nitrogen multiplied by 6.25, since the flesh bases have much higher percentages of nitrogen than are found in protein matters. Experience has shown that the complete extraction of dried meats by ether is difficult of accomplishment. After a few hours the extraction

⁷² Bureau of Chemistry, U. S. Department of Agriculture, Bulletin 107 (revised) 1912: 107-108.

should be interrupted, and the partially extracted sample finely ground in a mortar and again extracted. By proceeding in this manner it may be assumed that the total extract will represent the fat, although additional soluble matters are obtained by continuing the process. The methods which have been described in the preceding pages will be found sufficient for guidance in the examination of animal products and the analyst will find them, when modified to suit particular cases, adapted to the isolation and estimation of proximate food principles.

The methods of analyses given below⁷³ are those followed by Atwater and Woods except when otherwise stated.

Water and Water-Free Substance.—The drying is done in ordinary water ovens at a temperature of nominally 100°, but actually at 96° and 98°. For each analysis of animal tissues (flesh) one or more samples of from 50 to 100 grams of the freshly chopped substance are weighed on a small plate, heated for from 24 to 48 hours, cooled, allowed to stand in the open air for about 24 hours, weighed, ground, sifted through a sieve with circular holes one-half millimeter in diameter, bottled and set aside for analysis. In case of fat samples which cannot be worked through so fine a sieve, either a coarser sieve is used or the substance crushed as finely as practicable and bottled without sifting.

For the complete desiccation, a sample of about two grams of material is dried until it ceases to lose weight. It is extremely difficult to get an absolutely constant weight, though it is found that this is in most cases approximately attained in four hours.

Nitrogen, Protein, Albuminoids, etc.—The nitrogen is determined in the partly dried substance by the method of Kjeldahl. The protein is calculated by multiplying the percentage of nitrogen by 6.25. The nitrogenous matters in meats and fish, *i. e.*, in the materials which have practically no carbohydrates, are also estimated by subtracting the sum of ether extract and ash from the water-free substance, or the sum of water, ether extract and ash from the fresh substance, the remainder being taken as pro-

⁷³ Office of Experiment Stations, U. S. Dept. of Agri., Bulletin 29, 1896: 8.

teins, albuminoids, etc., by difference. While this is not absolutely correct measure of the total nitrogenous matter, it is doubtless more nearly so than the product of the nitrogen multiplied by 6.25.

Fat (Ether Extract).—The fat is extracted as directed under preserved meats.

Ash.—Ash is determined by the method recommended by the Association of Official Agricultural Chemists.

Fuel Value—Potential Energy.—The food materials are not necessarily burned in the calorimeter, but the fuel value of a pound of each of the foods, as given in the tables, is obtained by multiplying the number of hundredths of a pound of protein and of carbohydrates by 18.6 and the number of hundredths of a pound of fat by 42.2, and taking the sum of these three products as the number of calories of potential energy in the materials.

More reliable results are obtained by using the factors obtained by Stohmann; *viz.*, 5,731 calories for proteins, 9,500 calories for common glycerids, 9,231 calories for butter fat, 3,746 calories for pentose sugars, 3,749 calories for dextrose and levulose and 3,953 calories for sucrose and milk sugar.⁷⁴

578. Further Examination of Nitrogenous Bodies.—It is evident that both of the methods proposed above for the examination of the nitrogenous constituents of meats are unreliable. If the total nitrogen be determined and multiplied by 6.25 the product does not by any means represent the true quantity of nitrogenous matter since the flesh bases contain in some instances more than 25 per cent. of nitrogen.

If, on the other hand, the water, ash and fat in a meat sample be determined and the sum of their per cents. be subtracted from 100, the difference represents the nitrogenous bodies plus all undetermined matters and errors of analysis. The assumption that meats are free of carbohydrates is not tenable since glycogen is constantly found therein and in horse flesh in comparatively large amounts.

⁷⁴ Experiment Station Record, 1894-1895, 6 : 590, et seq.

If it be desired to study the nitrogenous bodies in detail the methods given by Leach⁷⁵ may be employed.

Aqueous extracts containing nitrogen are easily prepared for moist combustion by placing them in the digestion flasks, connecting the latter with the vacuum service and evaporating the contents of the flask nearly to dryness. The sulfuric acid is then added and the nitrogen converted into ammonia and determined in the usual manner.

ESTIMATION OF STARCH IN SAUSAGES.

579. Perrier's Method.—Perrier's Method of determining starch in sausages and similar bodies is as follows:⁷⁶

Five grams (if the starch content is low—less than two per cent.—which will be indicated by a preliminary test, use 10 grams) of finely divided sausage meat is placed in a 250 cubic centimeter flask with 100 cubic centimeters of water containing three cubic centimeters of hydrochloric acid. The flask is then placed in the autoclave. When the temperature has reached 120° it is maintained there for 40 minutes; then the flask is removed. Almost all the material is found to be dissolved. After cooling, the liquid is decanted upon a funnel, the neck of which is closed by asbestos or glass wool lightly packed, which will retain the solidified fat and the undissolved particles. The filtrate is received in a 200 cubic centimeter graduated flask. The residue remaining in the flask is washed twice with a little warm water, the wash water not being poured upon the funnel until it has been completely cooled.

The filtrate is then freed of the albuminoid substances which it contains by means of phosphotungstic acid in the presence of hydrochloric acid. To do this, add two cubic centimeters of concentrated hydrochloric acid and a slight excess of a 20 per cent. solution of phosphotungstic acid (10 cubic centimeters is usually sufficient). Under these conditions the precipitate which

⁷⁵ Leach, *Food Inspection and Analysis*, 2d ed. rev. and enl., 1909: 228-232.

⁷⁶ *Bulletin des Sciences Pharmacologiques*, 1908: 305.

is formed settles rapidly, and the supernatant liquid is clear and colorless. The volume is brought to 200 cubic centimeters with distilled water, and after agitation the liquid is filtered. In the filtered liquid the reducing sugar is determined by means of a Fehling solution.

580. Polarimetric Determination of Starch in Sausage Materials.—Lehmann and Schowalter have proposed a polarimetric method of determining starch in sausage which is carried out as follows:⁷⁷—To 27.5 grams of sausage meat add 80 cubic centimeters alcoholic potassium hydrate (100 grams KOH and 100 cubic centimeters H_2O per liter of 96 per cent. alcohol). After standing several hours heat the mixture on a water bath, decant, treat with 30 cubic centimeters more of the hydrate solution; filter, and wash with 50 per cent. alcohol. Dissolve the residue by heating with 25 cubic centimeters H_2O and five cubic centimeters double normal, NaOH, neutralize with normal HCl, heat and add 25 cubic centimeters of normal HCl. Heat on a water bath 10 minutes, cool, neutralize, add precipitated lead hydrate and make up to 100 cubic centimeters. Filter the solution, and polarize in a 200 millimeter tube. Each degree of the polarimetric scale is equivalent to one per cent. of pure starch.

581. Price's Modification.—Inasmuch as sugar is sometimes added to sausage, and at times certain sausages are made from meats that have been cured in pickling fluids containing sugar, together with the fact that reducing compounds other than starch naturally occur in the spices used in the preparation of sausages, the results obtained by the above method for estimating starch in meat food products can not be relied upon as representing the actual amount of starch present.

As Bigelow's modification of Mayrhofer's method has some good features, and the method proposed by Perrier also some, Price has combined the good features of the two methods with such modifications and additions as were thought necessary.

⁷⁷ Zeitschrift für Untersuchung der Nahrungs-und Gennssmittel, 1912, 24 : 319.

thereby obtaining a method in which the objectionable features were eliminated.⁷⁸

In a 200 cubic centimeter beaker treat 10 grams of finely divided meat with 75 cubic centimeters of an eight per cent. solution of potassium hydrate in 95 per cent. alcohol, and heat on the steam bath until all the meat is dissolved. This will require from 30 to 45 minutes. Add an equal volume of 95 per cent. alcohol cool, and allow to stand at least one hour. Filter by suction through a thin layer of asbestos in a Gooch crucible. Wash twice with warm four per cent. potassium hydrate in 50 per cent. alcohol and then twice with warm 50 per cent. alcohol. Discard the wash water. Endeavor to retain as much of the precipitate in the beaker as possible until the last washing. Place the crucible with contents in the original beaker, add 40 cubic centimeters of water and then 25 cubic centimeters of concentrated sulfuric acid. Stir during the addition of the acid and see that the acid comes in contact with all the precipitate. Allow to stand about five minutes, add 40 cubic centimeters of water, and heat just to boiling, stirring constantly. Transfer the solution to a 500 cubic centimeter graduated flask, add two cubic centimeters of a 20 per cent. aqueous solution of phosphotungstic acid, allow to cool to room temperature, and make up to mark with distilled water. Filter through a starch-free filter paper and determine the dextrose present in a 50 cubic centimeter portion of the filtrate with Fehling's solution after neutralizing the acid, using Low's method, for the determination of the copper in the cuprous oxid precipitate.⁷⁹ The amount of dextrose multiplied by 0.9 gives the equivalent in starch.

581a. Detection of Benzoic Acid in Meats.—By the illegal construction of the food law, allowing the use of benzoic acid and its salts in food products, it has become necessary, in testing meats for adulterations, to look for the presence of benzoic acid. This is accomplished as follows:^{79a}

⁷⁸ Bureau of Animal Industry, circular 203, 1912 : 5.

⁷⁹ Bureau of Chemistry, Bulletin 107 (revised), 1912 : 241.

^{79a} Zeitschrift für Untersuchung der Nahrungs und Genussmittel, 1913, 26 : 12-20. The Analyst, 1913, 38 : 414.

The method, which is due to Krüger, is based upon the well-known principle of distillation. Fifty grams of the finely divided meat are mixed with 45 cubic centimeters of 70 per cent. sulfuric acid and distilled in a current of steam until half a liter of the distillate is collected. The volume of liquid in the flask is kept constant during distillation. The distillate is filtered, rendered slightly alkaline with sodium hydroxid, and evaporated to a small volume. To the hot residual liquid potassium permanganate is added drop by drop until the pink coloration remains for at least five minutes. After this time the excess of the permanganate is destroyed by the addition of sodium sulfite, the mixture evaporated to 10 cubic centimeters transferred to a separating funnel, acidified with sulfuric, the containing basin rinsed with sodium sulfite solution and dilute sulfuric acid, and the washings added to the contents of the separating funnel. The whole solution, which should not measure more than 20 cubic centimeters is extracted with ether, the ethereal extract washed with water, the solvent allowed to evaporate spontaneously in a weighed dish, and the residue, after being dried for two hours over soda-lime, is weighed. This residue, which consists of benzoic acid is dissolved in alcohol, and the solution titrated with 1/10 normal hydroxid solution. This method is recommended as capable of extracting the whole of the benzoic acid from meats and other food products.

582. Detection of Horse Flesh.—Since horse flesh has become an important article of human food and is often sold as beef and sausage, a method of distinguishing it is desirable. The comparative anatomist is able to detect horse flesh when accompanied by its bones, or in portions sufficiently large for the identification of muscular characteristics. It is well known that fresh horse flesh contains a much higher percentage of glycogen than is found in other edible meats. Niebel has based a method of detecting horse flesh upon this fact, the glycogen being converted into dextrose and determined in the usual way. Whenever the percentage of reducing sugars in the dry fat-free flesh exceeds one per cent. Niebel infers that the sample under examination is

horse flesh.⁸⁰ It is now recognized, however, that glycogen is hydrolized rapidly after the death of the animal. Its absence in meat, therefore, can not be regarded as proof that horse flesh is not present.

The reaction for horse flesh, proposed by Bräutigam and Edelmann, is preferred by Baumert. In this test about 50 grams of the flesh are boiled for an hour with 200 cubic centimeters of water, the filtered bouillon evaporated to about half its volume, treated with dilute nitric acid and the clear filtrate covered with iodine water. Horse flesh, by reason of its high glycogen content, produces a burgundy-red zone at the points of contact of the two liquids. In the case of sausages, if starch have been added, a blue zone is produced, and if dextrin be present, a red zone, both of which obscure the glycogen reaction. The starch is easily removed by treating the bouillon with glacial acetic acid. No method is at present known for separating dextrin from glycogen. The detection of horse flesh is a matter of considerable importance to agriculture as well as to the consumers, especially of sausages. A considerable quantity of horse flesh is annually prepared in this country for export but it is believed that it is not marketed in this country. As a cheap substitute for beef and pork in sausages, its use must be regarded as fraudulent, although no objection can be urged against its sale when prepared from healthy slaughtered animals, when offered under its own name.⁸¹

The examination of the fat is also employed for the detection of horse flesh, as it has an iodine number of from 71 to 86 and its reading on the butyro refractometer is about 53°.7 at 40°.

METHODS OF DIGESTION

583. Artificial Digestion.—The nutrient values of cereals and other foods are determined both by chemical analysis and by digestion experiments. The heat forming properties of foods are disclosed by combustion in a calorimeter, but the quantity of

⁸⁰ *Zeitschrift für angewandte Chemie*, 1895 : 620.

⁸¹ *Zeitschrift für angewandte Chemie*, 1895 : 620.

heat produced is not in every case a guide to the ascertainment of the nutritive value. This is more certainly shown, especially in the case of protein bodies, by the action of the natural digestive ferments.

It is probable that the digestion, which is secured by the action of these ferments without the digestive organs, is not always the same as the natural process, but when the conditions which prevail in natural digestion are imitated as closely as possible the effects produced can be considered as approximately those of the alimentary canal in healthy action.

Three classes of ferments are active in artificial digestion, *viz.*, amylolytic ferments, serving to hydrolyze starch and sugars and to convert them into maltose, dextrose and levulose, lipolytic ferments, which decompose the fats and proteolytic ferments which act on the nitrogenous constituents of foods. When these ferments are made to act on foods under proper conditions of acidity and temperature, artificial digestion ensues, and by the measurement of the extent of the action an approximate estimate of their digestibility can be secured. In artificial digestion, the temperature should be kept near that of the body, *viz.*, at about 40°.

The soluble ferments which are active in the digestion of foods, as has been intimated, comprise three great classes. Among the first class, *viz.*, the amylolytic ferments, are included not only those which convert starch into maltose and finally dextrose, but also those which cause the hydrolysis of sugars in general. Among these may be mentioned ptyalin, invertase, emulsin, maltase, lactase, diastase, inulase, pectase and cytase ferments which act upon the celluloses and other fibers.

Among the lipolytic ferments, in addition to pancreatin which acts also upon protein and carbohydrate matter, may be mentioned a special one, lipase.

In the third class of ferments are found pepsin, trypsin or pancreatin and papain.

For further information in regard to the nature of the solu-

ble ferments and their nomenclature, the works of Bourquelot⁸² and Oppenheimer⁸³ may be consulted.

584. Amylolytic Ferments.—A very active ferment of this kind is found in the saliva. Saliva may be easily collected from school boys, who will be found willing to engage in its production if supplied with a chewing gum. A gum free of sugar is to be used, or if the chewing gum of commerce is employed, the saliva should not be collected until the sugar has disappeared. A dozen boys with vigorous chewing will soon provide a sufficient quantity of saliva for practical use. The amylolytic digestion is conducted in the apparatus hereinafter described for digestion with pepsin and pancreatin. The starch or sugar in fine powder is mixed with 10 parts of water and one part of saliva and kept at about 37°.5 for a definite time. The product is then examined for starch, sucrose, maltose, dextrose, dextrin and levulose by the processes already described. In natural digestion the hydrolysis of the carbohydrates is not completed in the mouth. The action of the ferment is somewhat diminished in the stomach, but not perhaps until half an hour after eating. The dilute hydrochloric acid in the stomach, which accumulates some time after eating, is not active in this hydrolysis. On the contrary the amylolytic ferment of the saliva is somewhat enfeebled by the presence of an acid. The active principle of the saliva is ptyalin.

The diastatic hydrolysis of starch has already been described (218). It is best secured at a somewhat higher temperature than that of the human stomach.

585. Lipolytic Ferments.—In the hydrolysis of glycerids in the process of digestion the fat acids and glycerol are set free. Whether the glycerids be completely hydrolyzed before absorption is not definitely known. In certain cases where large quantities of oil have been exhibited for remedial purposes, the fat acids and soaps have been found in spherical masses in the dejecta⁸⁴ and have been mistaken for gall stones.

⁸² Bourquelot, *Les Ferments Solubles (Diastases-Enzymes)*, 1896.

⁸³ Oppenheimer, *The Ferments and Their actions*, 1901.

⁸⁴ Wiley, *Medical News*, 1888, 53 : 95-96.

The fat which enters the chyle appears to be mostly unchanged, except that it is emulsified.⁸⁵ The lipolytic ferment can be prepared from the fresh pancreas, preferably from animals that have not been fed for 40 hours before killing. It is important to prepare the ferment entirely free of any trace of acid. The fresh glands are rubbed to a fine paste with powdered glass and extracted for four days with pure glycerol, to which one part of one per cent. soda solution has been added. The filtered liquor contains lipolytic, proteolytic and amylolytic ferments, and is employed for saponification by shaking with the fat to form an emulsion and keeping the mixture, with occasional shaking, at a temperature of from 40° to 60°. The free acids can be titrated or separated from the unsaponified fats by solution in alcohol.⁸⁶

Heretofore it has not been possible to separate a pure lipolytic ferment from any of the digestive glands. The digestion of carbohydrates and that of fats are intimately associated, and these two classes of foods seem to play nearly the same rôle in the animal economy.

The lipolytic ferments, prepared from the fresh pancreas, act also on the glucosids and other ester-like carbohydrate bodies. Since the fats may be regarded as ethers, the double action indicates the similarity of composition in the two classes of bodies.⁸⁷ The lipolytic ferments exist also in plants and have been isolated from rape seed.⁸⁸

586. Proteolytic Ferments.—The most important process in artificial digestion is the one relating to the action of the ferments on protein matters. The hydrolysis of fats and carbohydrates by natural ferments takes place best in an alkaline medium, while in the case of proteins when pepsin is used an acid medium is preferred. Since the acidity of the stomach is due chiefly to hydrochloric, that acid is employed in artificial digestion. The hydrolyte used is uniformly the natural ferment of the gastric

⁸⁵ Virchow's Archiv für pathologische Anatomie, 1891, **123** : 239.

⁸⁶ Landenberg, Handwörterbuch der Chemie, 1887, **4** : 122-123.

⁸⁷ Chemisches Centralblatt, 1892, **63** Bd. 2 : 579.

⁸⁸ Chemisches Centralblatt, 1890, **61** Bd. 2 : 628.

secretions, *viz.*, pepsin; but this is often followed by the pancreatic ferment, (pancreatin, trypsin) in an alkaline medium. During the digestion, the proteins are changed into proteoses, and peptones, and the measurement of this change determines the degree of digestion. The total protein matter is determined in the sample, and after the digestion is completed, the soluble products are removed by washing and the residual insoluble protein matter determined by moist combustion. The difference in the two determinations shows the quantity of protein matter digested. The investigations of Kühn on the digestion of proteins may be profitably consulted.⁸⁹ For a summary of digestion experiments in this country the résumé prepared by Jordan may be consulted.⁹⁰ The method used in the researches given below is fully described by Bigelow and Hamilton.⁹¹

587. Ferments Employed.—Both the pepsins of commerce and those prepared directly from the stomachs of pigs may be used. The commercial scale pepsin is found, as a rule, entirely satisfactory, and more uniform results are secured by its use than from pepsin solutions made from time to time from pig stomachs. In the preparation of the pepsin solution one gram of the best scale pepsin is dissolved in one liter of 0.33 per cent. hydrochloric acid. Two grams of the sample of food products, in fine powder, are suspended in 100 cubic centimeters of the solution and kept, with frequent shaking, at a temperature of 40° for 12 hours. The contents of the flask are poured on a wet filter, the residue on the filter well washed with water not above 40°, the filter paper and its contents transferred to a kjeldahl flask and the residual nitrogen determined and multiplied by 6.25 to get the undigested protein matter. A large number of digestions can be conducted at once in a bath shown in Fig. 110.⁹² The quantity of water in the bath should be as large as possible.

⁸⁹ Die Landwirtschaftlichen Versuchs-Stationen, 1894, **44** : 188.

Experiment Station Record, 1894-1895, **6** : 12.

⁹⁰ Experiment Station Record, 1894-1895, **6** : 5.

⁹¹ Journal of the American Chemical Society, 1894, **16** : 590, et seq.

⁹² From photograph made in the laboratory by Bigelow.

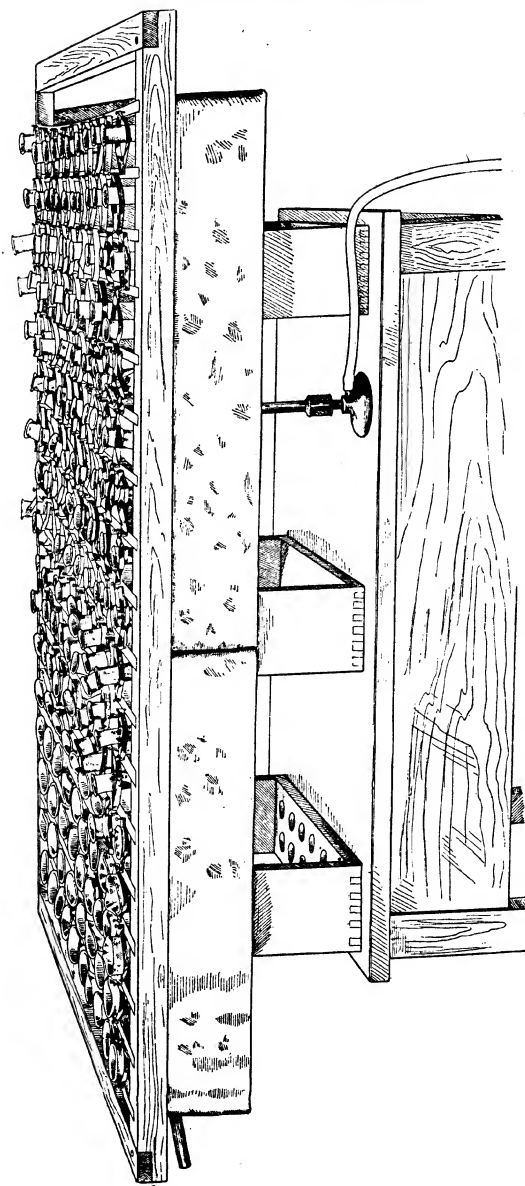


Fig. 110.—Bath for Artificial Digestion.

588. Digestion in Pepsin and Pancreatin.—The digestion of the proteins is not as a rule wholly accomplished by the stomach juices, and, therefore, in order to secure in artificial digestion results approximating those produced in the living organism, it is necessary to follow the treatment with pepsin by a similar one with the pancreas juices. The method employed is essentially that of Stutzer modified by Wilson.⁹³

The residue from the pepsin digestion, after washing, is treated for six hours at near 40° with 100 cubic centimeters of pancreas solution, prepared as follows:

Free the pancreas of a healthy steer of fat, pass it through a sausage grinder, rub one kilogram in a mortar with fine sand and allow to stand for a day or longer. Add three liters of lime water, one of glycerol, of 1.23 specific gravity, and a little chloroform and set aside for six days. Separate the liquor by pressure in a bag and filter it through paper. Before using, mix a quarter of a liter of the filtrate with three-quarters of a liter of water and five grams of dry sodium carbonate, or its equivalent crystallized, heat from 38° to 40° for two hours and filter.⁹⁴ In order to avoid the trouble of preparing the pancreas solution pure active pancreatin may be used.⁹⁵ One and a half grams of pure pancreatin and three grams of sodium carbonate are dissolved in one liter of water and 100 cubic centimeters of this solution are used for each two grams of the sample. In all cases where commercial pepsin and pancreatin are used, their activity should be tested with bodies such as boiled whites of eggs, whose coefficient of digestibility is well known and those samples be rejected which do not prove to have the required activity.⁹⁶

589. Digestion in Pancreas Extract.—In order to save the time required for successive digestions in pepsin and pancreatin Nieb-

⁹³ Journal of the Society of Chemical Industry, 1891, 10 : 118.

⁹⁴ Die Landwirtschaftlichen Versuchs-Stationen, 1889, 36 : 321, et. seq. Chemical Division, U. S. Department of Agriculture, Bulletin 13,

1893 : 1028.

⁹⁵ Wilson, Journal of the Society of Chemical Industry, 1891, 10 : 118.

⁹⁶ U. S. Dispensatory, 1907, 19th Ed. : 921.

ling has proposed to make the digestion in the pancreas extract alone.⁹⁷ This process and also a slight modification of it have been used with success by Bigelow and McElroy.⁹⁸ Two grams of the sample are washed with ether and placed in a digestion flask with 100 cubic centimeters of two-tenths per cent. hydrochloric acid. The contents of the flask are boiled for 15 minutes, cooled, and made slightly alkaline with sodium carbonate. One hundred cubic centimeters of the unfiltered pancreas solution, prepared as directed above, are added and the digestion continued at 40° for six hours. The residue is thrown on a filter, washed, and the nitrogen determined. The method is simplified by the substitution of active commercial pancreatin for pancreas extract. The solution of the ferment is made of the same strength as is specified above.

590. Artificial Digestion of Cheese.—The artificial digestion of cheese is conducted by Stutzer as follows:⁹⁹

The digestive liquor is prepared from the fresh stomachs of pigs by cutting them into fine pieces and mixing with five liters of water and 100 cubic centimeters of hydrochloric acid for each stomach. To prevent decomposition, two and a half grams of thymol, previously dissolved in alcohol, are added to each 600 cubic centimeters of the mixture. The mixture is allowed to stand for a day with occasional shaking, poured into a flannel bag and the liquid portion allowed to drain without pressing. The liquor obtained in this way is filtered, first through coarse and then through fine paper, and when thus prepared will keep several months without change. It is advisable to determine the content of hydrochloric acid in the liquor by titration and this content should be two-tenths of a per cent. The cheese to be digested is mixed with sand as previously described, freed of fat by extraction with ether, and a quantity corresponding to five grams of cheese placed in a beaker, covered with half a liter of the digestive liquor and kept at a temperature of 40° for 48 hours.

⁹⁷ *Landwirtschaftliche Jahrbücher*, 1890, 19 : 149.

⁹⁸ Chemical Division, U. S. Department of Agriculture, Bulletin 13, 1893 : 1028.

⁹⁹ *Zeitschrift für analytische chemie*, 1896, 35 : 497-498.

At intervals of two hours the flasks are well shaken and five cubic centimeters of a 10 per cent. solution of hydrochloric acid added and this treatment continued until the quantity of hydrochloric acid amounts to one per cent. After the digestion is finished, the contents of the beaker are thrown on a filter, washed with water and the nitrogen determined in the usual way in the residue. By allowing the pepsin solution to act for two days as described above, the subsequent digestion with pancreas solution is superfluous.

591. Suggestions Regarding Manipulation.—The filter papers should be as quick working as possible to secure the separation of all undissolved particles. They should be of sufficient size to hold the whole contents of the digestion flask at once, since if allowed to become empty and partially dry, filtration is greatly impeded. The residue should be dried at once if not submitted immediately to moist combustion. After drying, the determination of the nitrogen can be made at any convenient time. Beaker flasks, *i. e.*, lip Erlenmeyers with a wide mouth are most convenient for holding the materials during digestion. The flasks are most conveniently held by a crossed rubber band attached at either end to pins in the wooden slats extending across the digestive bath. The bath should be suspended by cords from supports on the ceiling and a gentle rotatory motion imparted to it resembling the peristaltic action attending natural digestion.

592. Natural Digestion.—The digestion of foods by natural processes is determined chiefly by the classes of ferments already noted. The principle underlying digestive experiments with the animal organism may be stated as follows: A given weight of food of known composition is fed to a healthy animal under the conditions of careful control and preparation already mentioned. The solid dejecta of the animal during a given period are collected and weighed daily, being received directly from the animal in an appropriate bag, safely secured, as is shown in the accompanying figure. The dejecta are weighed, dried, ground to a fine powder, mixed and a representative part analyzed. The difference between the solid bodies in the dejecta and those given

in the food during the period of experiment represents those nutrients which have been digested and absorbed during the passage of the food through the alimentary canal. The urine, containing solid bodies representing the waste of the animal organism, does not require to be analyzed for the simple control of digestive activity outlined above. In a complete determination of this kind the exhalations from the surface of the body and from the lungs are also determined. In the latter case the human animal is selected for the experiment; in the former it is more convenient to employ the lower animals, such as the sheep and cow.

The arrangement of the stalls and of the apparatus for collecting the excreta should be such as is both convenient and effective.¹

The method of constructing a bag for attachment to a sheep is shown in Fig. 111. It is made according to the directions given by Gay, of heavy cloth and in such a way as to fit closely the posterior parts of the animal.² When attached, its appearance is shown in Fig. 112.

Healthy animals in the prime of life are used, and the feeding experiments are conducted with as large a number of animals as possible, in order to eliminate the effects of idiosyncrasy. The food used is previously prepared in abundant quantity and its composition determined by the analysis of an average sample.

The feeding period is divided into two parts. In the first part the animal is fed for a few days with the selected food until it is certain that all the excreta are derived from the nutrients used. In the second part the same food is continued and the excreta collected, weighed, the moisture determined, and the total weight of the water-free excreta ascertained. The first part should be of at least seven and the second of at least five days duration. The urine and dung are analyzed separately. Males are preferred for the digestion experiments because of the greater ease

¹ Maine Agricultural Experiment Station, Annual Report, 1891, pt. 2: 25-28.

Gay, *Annales Agronomiques*, 1896, 22 : 145 et seq.

² *Annales Agronomiques*, 1895, 21 : 149-150.

of collecting the urine and feces without mixing. For ordinary purposes the feces only are collected. The methods of analysis do not differ from those described for the determination of the usual ingredients of a food.

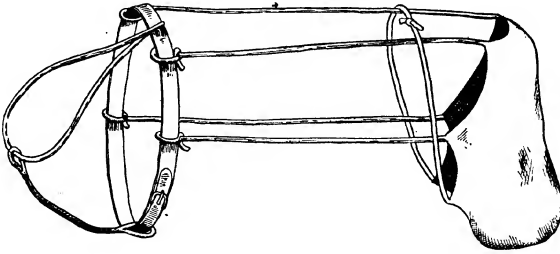


Fig. 111.—Bag for Collecting Feces.



Fig. 112.—Fecal Bag Attachment.

Example.—The following data taken from the results of digestive experiments, obtained at the Maine Station, will illus-

trate the method of comparing the composition of the food with that of the feces and of determining the degree of digestion which the proteins and other constituents of the food have undergone.

COMPOSITION OF MAIZE FODDER AND OF FECES THEREFROM AFTER FEEDING TO SHEEP.

BEFORE DRYING.

Food.	Water, per cent.	Ash, per cent.	Protein, per cent.	Fiber per cent.	Fat, per cent.	Undeter- mined, per cent.
Sweet maize.....	83.85	1.13	2.18	4.14	0.62	8.08
Feces	72.01

DRY.

Food.	Ash, per cent.	Protein, per cent.	Fiber, per cent.	Fat, per cent.	Undeter- mined, per cent.
Sweet maize.....	7.01	13.52	25.63	3.86	49.93
Feces	14.42	17.52	19.34	2.68	46.04

DAILY WEIGHTS.

Food.	Green, grams.	Dry, grams.
Sweet maize	2521	407
Feces	445	125

PER CENT. DIGESTED.

Food.	Ash.	Protein.	Fiber.	Undeter- mined.	Fat.
Sweet maize.....	37.0	60.2	76.9	71.8	78.3

In the above instance it is seen that the coefficient of digestibility extended from 37.0 per cent. in the case of the mineral components of the food, to 78.3 per cent. in the case of the fats. These data are taken only from the results obtained from a single sheep and one article of food. The mean data secured from two animals and three kinds of maize fodder show the following per cent. of digestibility: Ash 39.4, protein 61.8, fiber 76.7, undetermined matters 72.1, fat 76.4. The undetermined matters are those usually known as nitrogen free extract and composed chiefly of pentosans and other carbohydrates.³

593. Natural Digestibility of Pentosans.—The digestibility of pentosan bodies in foods under the influence of natural fer-

³ Maine Agricultural Experiment Station, Annual Report, 1891, pt. 2: 53.

ments has been investigated by Lindsey and Holland.⁴ The feeding and collection of the feces is carried on as described above and the relative proportions of pentosan bodies in the foods and feces determined by estimating the furfuraldehyd as prescribed in paragraphs 194-199.⁵

PRESERVED MEATS.

594. Methods of Examination.—In general the methods of examination are the same as those applied in the study of fresh meats. The contents of water, salt and other preservatives, fat and nitrogenous matters are of most importance. The preserved meats are run through meat cutters until reduced to a fine pulp. Most potted meats are already in a state of subdivision well suited to analytical work but should be run through a sausage grinder to secure a thorough and uniform mixture. The composition of preserved meats has been thoroughly studied in laboratory work by Davis and Bigelow.⁶

595. Estimation of Fat.—Attention has already been called to the difficulty of extracting the fat from meats by ether or other solvents.⁷ In preserved meats, as well as in fresh, it is preferable to adopt some method which will permit of the decomposition of the other organic matters and the separation of the fat in a free state.

Dormeyer⁸ has found that much fat remained undissolved after a month's extraction with ether in a Soxhlet apparatus, and that the preliminary removal of protein by pepsin digestion made it possible to obtain an additional amount of fat averaging 8.5 per cent. of the total quantity.

Oswaldo Polimanti⁹ found that by shaking two grams of

⁴ Massachusetts Agricultural Experiment Station, Twelfth Annual Report, 1894 : 175.

⁵ See also paragraph —, this volume.

⁶ Division of Chemistry, U. S. Department Agriculture, Bulletin 13, pt. 10, 1902.

⁷ Vid. this volume, paragraph 577.

⁸ Archiv für die gesammte Physiologie, 1895, 61 : 341-342.

Archiv für die gesammte Physiologie, 1897, 65 : 90-108.

⁹ Archiv für die gesammte Physiologie, 1898, 70 : 366.

powdered meat for six hours with 200 cubic centimeters of ether and two cubic centimeters of mercury and weighing the residue obtained by the evaporation of an aliquot portion of the extract he could obtain the same result as by Dormeyer's method.

Liebermann and Székely¹⁰ saponify the fat by means of potassium hydroxid, add alcohol, acidify carefully with sulfuric acid, extract with petroleum ether and determine the fatty acids by titration. The fats are calculated by formulæ given by the authors. The method is accurate and has the advantage of great rapidity.

Tangl and Weiser¹¹ obtained slightly higher results by this method than by Dormeyer's gastric digestion method.

The most promising methods are those employed in milk analyses for the solution of nitrogenous matters. Sulfuric or hydrochloric acid may be used for this purpose, preference being given to sulfuric. The separated fats may be taken up with ether or separated by centrifugal action. A method of this kind for preserved meats is described below.

About six grams of the moist preserved meat are placed in a calibrated test tube and dissolved in 25 cubic centimeters of fuming hydrochloric acid. The tube is placed in a water bath, quickly heated to boiling and kept at that temperature for half an hour. About 20 cubic centimeters of cold water are added and the temperature lowered to 30°, then 20 cubic centimeters of ether and the tube gently shaken to promote the solution of the fat. When the ether layer has separated, its volume is read and an aliquot part removed by means of a pipette, dried and weighed. The separation of the ethereal solution is greatly promoted by whirling.

Kila¹² employs a modification of the Babcock method for the estimation of fat in milk. Two and a half or five grams of meat (according to the fat content) are placed in a tube of Gerber's acid butyrometer and dissolved in 8.5 or 17 cubic centimeters

¹⁰ Archiv für die gesammte Physiologie, 1898, **72** : 360-366.

¹¹ Archiv für die gasammte Physiologie, 1898, **72** : 367-369.

¹² Archiv für Hygiene, 1894, **51** : 165-178.

respectively of 1:1 sulfuric acid at the temperature of from 60° to 70°. One cubic centimeter of amyl alcohol is added and warm dilute sulfuric acid till the fat layer is within the scale of the tube. The tube is then whirled in the centrifuge for from three to five minutes and the amount of fat read on the scale.

Examination of Extracted Fat.—By the examination of the fat extracted from preserved meat valuable information regarding its nature and origin may be obtained. The meat is shaken repeatedly during several hours with low boiling petroleum ether. The petroleum ether is then poured off, removed from the dissolved fat as far as practicable by distillation and the last traces of the solvent separated by means of a vacuum desiccator. The residual fat is then examined by the methods given on page 000.

596. Meat Preservatives.—Various bodies are used to give taste and color to preserved meats and to preserve them from fermentation. The most important of these bodies are common salt, potassium and sodium nitrates, sulfurous, boric, benzoic and salicylic acids, formaldehyd and saccharin. For the methods of detecting and isolating these bodies the official methods may be consulted.¹³

DETERMINATION OF NUTRITIVE VALUES.

597. Nutritive Value of Foods.—The value of a food as a nutrient depends on the amount of heat it gives on combustion in the tissues of the body, *i. e.*, oxidation, and in its fitness to nourish the tissues of the body, to promote growth and repair waste. The foods which supply heat to the body are organic in their nature and are typically represented by fats and carbohydrates. The foods which promote growth and supply waste are not only those which pre-eminently supply heat, but also include the mineral bodies and organic nitrogenous matters represented typically by the proteins and compounds of lime and phosphorus. It is not proper to say that one class of food is definitely devoted to heat forming and another to tissue building, inasmuch as the same substance may play an important rôle in both directions.

¹³ Bureau of Chemistry, Bulletin 107 (revised), 1912: 111-112.

As heat formers, carbohydrates and proteins have an almost equal value, as measured by combustion in the tissues, while fat has a double value for this purpose. The assumption that combustion in oxygen forms a just criterion for determining the value of a food must not be taken too literally. There are only a few bodies of the vast number which burn in oxygen that are capable of assimilation and oxidation by the animal organism. Only those parts of the food that become soluble and assimilable under the action of the digestive ferments, take part in nutrition and the percentage of food materials digested varies within wide limits but rarely approaches 100. We have no means of knowing how far the decomposition (oxidation) extends before assimilation, and therefore no theoretical means of calculating the quantity of heat which is produced during the progress of digestion. The vital thermostat is far more delicate than any mechanical contrivance for regulating temperature and the quantity of food, in a state of health, converted into heat, is just sufficient to maintain the temperature of the body at a normal degree. Any excess of heat produced, as by violent muscular exertion, is dissipated through the lungs, the perspiration and other secretions of the body.

Pure cellulose or undigestible fiber, when burned in oxygen, will give a thermal value approximating that of sugar, but no illustration is required to show that when taken into the system the bodily heat afforded by it is insignificant in quantity.

Thermal values determined outside of the body, therefore, have only a comparative usefulness in determining nutritive worth, except when applied to foods of approximately the same digestive coefficient.

598. Comparative Value of Food Constituents.—It has already been noted that, judged by combustion in oxygen, carbohydrates have less than half the thermal value possessed by fats and proteins a considerably higher thermal factor. Commercially, the values of foods depend in a far greater degree on their flavor and cooking qualities than upon the amount of nutrition they contain. Butter fat, which is scarcely more nutritious than tallow, is worth twice as much in the market, while the prices

paid for vegetables and fruits are not based to any great extent on their food properties.¹⁴ In cereals, especially in wheat, the quantity of fat is relatively small, and starch and protein are the preponderating elements. In meats, carbohydrates are practically eliminated and fats and proteins are the predominating constituents.

In the markets, fats and proteins command far higher prices than sugars and starches. The relative commercial food value of a cereal may be roughly approximated by multiplying the percentages of fat and protein by two and a half and adding the products to the percentage of carbohydrates less insoluble fiber. This method was adopted in valuing the cereals at the World's Columbian Exposition.¹⁵

599. Nutritive Ratio.—In most foods the nutritive ratio is that existing between the percentage of proteins and that of carbohydrates, increased by multiplying the fat by two and a quarter and adding the product. In a cereal containing 12 per cent. of protein, 72 of carbohydrates, exclusive of fiber, and three of fat, the ratio is $12:72 + 3 \times 2.25 = 6.56$. Instead of calculating the nutritive ratio directly from the data obtained by analysis, it may be reckoned from the per cents. of the three substances in the sample multiplied by their digestive coefficient. Since the relative amounts of proteins, fats and carbohydrates digested do not greatly differ, the numerical expression of the nutritive ratio is nearly the same when obtained by each of these methods of calculation.

Where the proportion of protein is relatively large the ratio is called narrow, 1:4....6. When the proportion of protein is relatively small the ratio is called wide, 1:8....12. In feeding, the nutritive ratio is varied in harmony with the purpose in view, a narrow ratio favoring the development of muscular tissue, and a wide one promoting the deposition of fat and the development of heat. These principles guide the scientific farmer in

¹⁴ Chemical Division, U. S. Department of Agriculture, Bulletin 13, 1893: 1020.

¹⁵ Chemical Division, U. S. Department of Agriculture, Bulletin 45, 1895: 12.

mixing rations for his stock, the work horses receiving a comparatively narrow and the bees a relatively wide ratio in their food. On the other hand, milch cows should be fed a comparatively high nitrogenous ration since protein is essential to the production of milk.

600. Calorimetric Analyses of Foods.—The general principles of calorimetry have been already noticed. The theoretical and chemical relations of calorimetry have been fully discussed by Berthelot, Thomsen, Ostwald and Muir.¹⁶ In the analyses of foods the values as determined by calculation or combustion are of importance in determining the nutritive relations.

Atwater has presented a résumé of the history and importance of the calorimetric investigations of foods to which the analyst is referred.¹⁷

In the computation of food values the percentages of proteins, carbohydrates and fats are determined and the required data obtained by applying the factors 4,000, 5,700 and 9,300 calories for one gram of carbohydrates, proteins and fats respectively.

For most purposes the computed values are sufficient, but it is well to check them from time to time by actual combustions in a calorimeter.

601. Combustion in Oxygen.—The author made a series of combustions of carbonaceous materials in oxygen at the laboratory of Purdue University in 1877, the ignition being secured by a platinum wire rendered incandescent by the electric current. The data obtained were unsatisfactory on account of the crudeness of the apparatus. The discovery of the process of burning the samples in oxygen at a high pressure has made it possible to get expressions of thermal data which while not yet perfect, possess a working degree of accuracy. The best form of bomb

¹⁶ Berthelot, *Essai de mecanique, chimie*, 1879.

Thomsen, *Thermo-Chemische Untersuchungen*, 1882.

Ostwald, *Allgemeine Chemie*, 2, ungearb. Aufl., 1903.

Muir, *Elements of Thermal Chemistry*, 1885.

¹⁷ Office of Experiment Stations, U. S. Department of Agriculture, Bulletin 21, 1895 : 113 et. seq.

Connecticut (Storrs) Agricultural Experiment Station, Seventh Annual Report, 1894 : 135, et. seq.

calorimeter heretofore employed is that of Hempel, as modified by Atwater and Woods.¹⁸

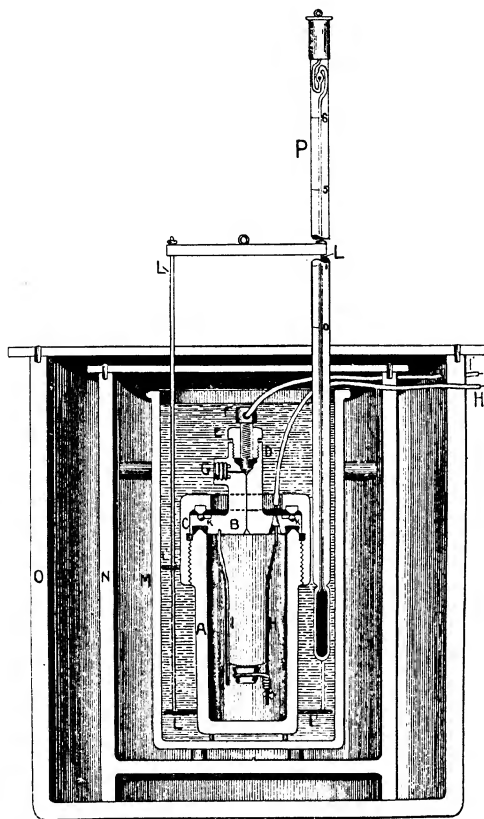


Fig. 113.—Hempel and Atwater's Calorimeter.

A section of this calorimeter, with all the parts in place, is shown in Fig. 113.

In the figure the steel cylinder A, about 12.5 centimeters deep

¹⁸ Office of Experiment Stations, U. S. Department of Agriculture, Bulletin 21, 1895: 123 et. seq.

Connecticut (Storrs) Agricultural Experiment Station, Seventh Annual Report, 1894: 135 et. seq.

and 6.2 in diameter, represents the chamber in which the combustion takes place. Its walls are about half a centimeter thick and it weighs about three kilograms. It is closed, when all the parts are ready and the sample in place, by the collar C, which is secured gas tight by means of a powerful spanner. The cover is provided with a neck D carrying a screw E and a valve screw F. In the neck D, where the bottom of the cylinder screw E rests, is a shoulder fitted with a lead washer. Through G the oxygen used for combustion is introduced. The upper edge of the cylinder A is beveled and fits into a groove in the cover B, carrying a soft metal washer. To facilitate the screwing on of the cover, ball bearings KK, made of hard steel, are introduced between the collar and the cover. The platinum wires H and I support the platinum crucible holding the combustible bodies which are ignited by raising the spiral iron wire connecting them to the temperature of fusion by an electric current. The combustion apparatus when charged is immersed in a metal cylinder M, containing water and resting on small cylinders of cork. The water is stirred by the apparatus LL. The cylinder M is contained in two large concentric cylinders, N, O, made of non-conducting materials and covered with disks of hard rubber. The space between O and N may be filled with water. The temperature is measured by the thermometer P, graduated to hundredths of a degree and the reading is best accomplished by means of a cathetometer.

602. The Williams Calorimeter.—The calorimeter bomb has been improved by Williams by making it of aluminum bronze of a spheroidal shape. The interior of the bomb is plated with gold. By an ingenious arrangement of contacts the firing is secured by means of a permanently insulated electrode fixed in the side of the bomb. The calorimetric water, as well as that in the insulating vessel, is stirred by means of an electrical screw so regulated as to produce no appreciable degree of heat mechanically. The combustion is started by fusing a fine platinum wire of definite length and thickness by means of an electric current. The heat value of this fusion is determined and the calories produced deducted from the total calories of the combustion. The valve

admitting the oxygen is sealed automatically on breaking connection with the oxygen cylinder. The effluent gases, at the end of the combustion, may be withdrawn through an alkaline solution and any nitric acid therein thus be fixed and determined.¹⁹

603. Manipulation and Calculation.—The material to be burned is conveniently prepared by pressing it into tablets. The oxygen is supplied from cylinders, of which two should be used, one at a pressure of more than 20 atmospheres. By this arrangement a pump is not required.

In practical use, a known weight of the substance to be burned is placed in the platinum capsule, the cover of the bomb screwed on, after all adjustments have been made, and the apparatus immersed in the water contained in M, which should be about 2° below room temperature. All the covers are placed in position and the temperature of the water in M begins to rise. Readings of the thermometer are taken at intervals of about one minute for six minutes, at which time the temperature of the bomb and calorimetric water may be regarded as sensibly the same. The electric current is turned on, the iron wire at once melts, ignites the substance and the combustion rapidly takes place. In the case of bodies which do not burn readily Atwater adds to them some naphthalene, the thermal value of which is previously determined. The calories due to the combustion of the added naphthalene are deducted from the total calories obtained.

The temperature of the water in M rises rapidly at first, and readings are made at intervals of one minute for five minutes, and then again after 10 minutes. The first of the initial readings, the one at the moment of turning on the current, and the last one mentioned above are the data from which the correction, made necessary by the influence of the temperature of the room, is calculated by the following formulas.²⁰

The preliminary readings of the thermometer at one minute intervals are represented by $t_1, t_2, t_3 \dots t_{n_1}$. The last observation t_{n_1} is taken as the beginning temperature of the combustion and is

¹⁹ From personal inspection by author in Williams' laboratory, 161 Tremont St., Boston, Mass.

²⁰ Journal für praktische Chemie, 1889 : 147, Neue Folge, 39 : 517 et seq.

represented in the formulas for calculations by θ_1 . The readings after combustion are also made at intervals of one minute, and are designated by $\theta_2, \theta_3 \dots \theta_n$. The readings are continued until there is no observed change between the last two. Generally this is secured by five or six readings.

The third period of observations begins with the last reading θ_n , which in the next series is represented by $t'_1, t'_2 \dots t'_{n_2}$.

In order to make the formulas less cumbersome let

$$\begin{aligned}\frac{t_{n_1} - t_1}{n_1 - 1} &= v, \\ \frac{t'_{n_2} - t'_1}{n_2 - 1} &= v', \\ \frac{t_1 + t_2 + t_3 \dots t_{n_1}}{n_1} &= t, \\ \text{and } \frac{t'_1 + t'_2 + t'_3 \dots t'_{n_2}}{n_2} &= t'.\end{aligned}$$

The correction to be made to the difference between $\theta_n - \theta_1$ for the influence of the outside temperature is determined by the formula of Regnault-Pfaundler, which is as follows:

$$\Sigma \Delta t = \frac{v - v'}{t' - t} \left(\sum_1^{n-1} \theta r + \frac{\theta_n + \theta_1}{2} - nt \right) - (n - 1) v,$$

in which $\sum_1^{n-1} \theta r$ is calculated from the observation of the thermometer θ_1, θ_2 etc., made immediately after the combustion. It is equal to the sum of observations θ_1, θ_2 etc., increased by an arbitrary factor equivalent to $\frac{\theta_2 - \theta_1}{9}$, which is made neces-

sary by reason of the irregularity of the temperature increase during the first minute after combustion, the mean temperature during that minute being somewhat higher than the mean of the temperatures at the commencement and end of that time.

The quantity of heat formed by the combustion of the iron wire used for igniting the sample is to be deducted from the total heat produced. This correction may be determined once for all, the weight of the iron wire used being noted and that of any unburned portion being ascertained after the combustion.

Ten milligrams of iron, on complete combustion, will give 16 calories.

In the combustion of substances containing nitrogen, or in case the free nitrogen of the air be not wholly expelled from the apparatus before the burning, nitric acid is formed which is dissolved by the water produced.

The heat produced by the solution of nitric acid in water is 14.3 Calories per gram molecule. The quantity of nitric acid formed is determined by titration and a corresponding reduction made in the total calculated calories.

In the titration of nitric acid it is advisable to make use of an alkaline solution, of which one liter is equivalent to 4.406 grams of nitric acid. One cubic centimeter of the reagent is equivalent to a quantity of nitric acid represented by one calorie.

Since the materials of which the bomb is composed have a specific heat different from that of water, it is necessary to compute the water thermal value of each apparatus.

The hydrothermal equivalent of the whole apparatus is most simply determined by immersing it at a given temperature in water of a different temperature.²¹ With small apparatus this method is quite sufficient, but there are many difficulties attending its application to large systems weighing several kilograms. In these cases the hydrothermal equivalent may be calculated from the specific heats of the various components of the apparatus.

In calculating these values the specific heats of the various components of the apparatus are as follows:

Brass	0.093
Steel	0.1097
Platinum	0.0324
Copper	0.09245
Lead	0.0315
Oxygen	0.2189
Glass	0.190
Mercury	0.0332
Hard rubber	0.33125

²¹ Berthelot, *Annales de Chimie et de Physique*, 1887, 6^e Serie, 10 : 439.

Example.—It is required to calculate the hydrothermal value of a calorimeter composed of the following substances :

	Hydrothermal value,
Steel bomb and cover, 2,850 grams \times 0.1097.....	312.65 grams.
Platinum lining, capsule and wires, 120 grams \times 0.0324....	3.89 "
Lead washer, 100 grams \times 0.0315.....	3.15 "
Brass outer cylinder, 500 grams \times 0.093.....	46.50 "
Mercury in thermometer, 10 grams \times 0.0332	0.33 "
Glass (part of thermometer in water), 10 grams \times 0.19	1.90 "
Brass stirring apparatus (part in water), 100 grams \times 0.093..	9.30 "
Total water value of system	377.72 "

When a bomb of 300 cubic centimeters capacity is filled with oxygen at a pressure of 24 atmospheres it will hold about 10 grams of the gas, equivalent to a water value of 2.40 grams. Hence the water value of the above system when charged, assuming the bomb to be of the capacity mentioned, is 380.12 grams.

If the cylinder holding the water be made of fiber or other non-conducting substance, its specific heat is best determined by filling it in a known temperature with water at a definite different temperature.

It is advisable to have the water cylinder of such a size as to permit the use of a quantity of water for the total immersion of the bomb which will weigh, with the water value of the apparatus, an even number of grams. In the case above, 2622.28 grams of water placed in the cylinder will make a water value of 3,000 grams, which is one quite convenient for calculation.

604. Computing the Calories of Combustion.—In the preceding paragraph has been given a brief account of the construction of the calorimeter and of the methods of standardizing it and securing the necessary corrections in the data directly obtained in its use. An illustration of the details of computing the calories of combustion taken from the paper of Stohmann, Kleber and Langbein, will be a sufficient guide for the analyst in conducting the combustion and in the use of the data obtained.²²

²² Journal für praktische chemie, 1889, 147 ; Neue Folge, 39 : 522-523.

The data in paragraph 603 are taken from Stohmann, Zeitschrift für Biologie, 1895, 31 : 364, and Experiment Station Record, 1894-1895, 6 : 590.

Weight of substance burned, 1.07 grams.

Water value of system (water + apparatus), 2,500 grams.

Preliminary thermometric readings, $t_1 = 26.8$; $t_2 = 27.2$; $t_3 = 27.7$; $t_4 = 28.1$; $t_5 = 28.5$; $t_{n_1} = 28.9$.

Thermometric reading after combustion, $\theta_1 = 28.9$; $\theta_2 = 202$; $\theta_3 = 213$; $\theta_4 = 214.2$; $\theta_n = 214.0$.

Final thermometric readings, $t'_1 = 214.0$; $t'_2 = 213.8$; $t'_3 = 213.6$; $t'_4 = 213.5$; $t'_5 = 213.3$; $t'_6 = 213.1$; $t'_7 = 212.9$; $t'_8 = 212.7$; $t'_9 = 212.6$; $t'_{10} = 212.4$; $t_{n_2} = 212.2$.

From the formulas given above the following numerical values are computed :

$$v = 0.42.$$

$$v' = -0.18.$$

$$t = 27.9.$$

$$t' = 213.1.$$

$$n = 5.$$

$$\sum_{i=1}^{n-1} \theta r = \theta_1 + \theta_2 + \theta_3 + \theta_4 + \frac{\theta_2 - \theta_1}{9} = 677.$$

Substituting these values in the formula of Regnault-Pfaundler, the value of the correction for the influence of the external air is :

$$\Sigma \Delta t = \left[\frac{0.42 - (-0.18)}{213.1 - 27.9} \left(677 + \frac{214 + 29}{2} - (5 \times 27.9) \right) - (4 \times 0.42) \right] = 0.45, \text{ which is to be added to the end temperature } (\theta_n = 214.0).$$

The computation is then made from the following data :

Corrected end temperature ($\theta_n + 0.45$).....	214.45 = 15°.3699
Beginning temperature (θ_1).....	28.90 = 12°.8406
Increase in temperature.....	185.55 = 2°.5293
Total calories 2.5293×2500	= 6323.3
Of which there were due to iron burned ...	9.1
“ “ “ nitric acid dissolved ...	8.2
Total calories due to substance.....	6316.0

The thermometric readings are given in the divisions of the thermometer which in this case are so adjusted as to have the number 28.90 correspond to 12°.8406, and each division is nearly equivalent to 0°.0136 thermometric degree.

The number of calories above given is the proper one when the computation is made to refer to constant volume. By reason of the consumption of oxygen and the change of temperature, although mutually compensatory, the pressure may be changed at the end of the operation. The conversion of the data obtained at constant volume referred to constant pressure may be made by the following formula, in which $[Q]$ represents the calories from constant volume and Q the desired data for constant pressure, O the number of oxygen atoms, H the number of hydrogen atoms in a molecule of the substance, and 0.291 a constant for a temperature of about 18° , at which the observations should be made.

$$Q = [Q] + \left(\frac{H}{2} - O \right) 0.291.$$

605. Simplified Corrections.—An adiabatic calorimeter has been devised by Benedict and Higgins,²³ in which the media surrounding it are always kept at the same temperature as the calorimeter itself. With this instrument the elaborate, time consuming warming and cooling corrections are eliminated as are also the periods of observation before the combustion and after maximum temperature is reached. With this instrument the rise in temperature following combustion is multiplied by the weight of water plus the water equivalent of the calorimeter, and the product, after deducting the ignition heat and heat of combustion of nitrogen to nitric acid, is divided by the weight of the substance. The operation and calculation are thus greatly simplified and more accurate results are secured.

606. Calorimetric Equivalent.—By the term 'calorie' is understood the quantity of heat required to raise one gram of water, at an initial temperature of about 18° , one degree. The term 'Calorie' denotes the quantity of heat, in like conditions, required to raise one kilogram of water one degree.

For purposes of comparison and for assisting the analyst in adjusting his apparatus so as to give reliable results, the follow-

²³ Journal American Chemical Society, 1910, 32 : 461.

ing data, giving the calories per gram of some common food materials, are given:

Substance	Calories	Chemical composition				
		C.	H.	N.	S.	O.
Proteins		Per cent.	Percent.	Percent.	Percent.	Percent.
Serum albumin.....	5917.8	53.93	7.65	15.15	1.18	22.09
Casein	5867.0	54.02	7.33	15.52	0.75	22.38
Egg albumin	5735.0	52.95	7.50	15.19	1.51	22.85
Meat free of fat and ex- tracted with water	5720.0	52.11	6.76	18.14	0.96	22.66
Peptone	5298.8	50.10	6.45	16.42	1.24	25.79
Proteins (mean).....	5730.8	52.71	7.09	16.02	1.03	23.15
Glycerids						
Butter fat.....	9325.0	—	—	—	—	—
Linseed oil.....	9488.0	—	—	—	—	—
Olive oil.....	9467.0	—	—	—	—	—
Carbohydrates		Formula				
Arabinose	3722.0	$C_5H_{10}O_5$				
Xylose	3746.0	$C_5H_{10}O_5$				
Dextrose	3742.6	$C_6H_{12}O_6$				
Levulose	3755.0	$C_6H_{12}O_6$				
Sucrose	3957.0	$C_{12}H_{22}O_{11}$				
Lactose	3736.8	$C_{12}H_{22}O_{11} + H_2O$				
Maltose	3949.3	$C_{12}H_{22}O_{11}$				

607. Distinction between Butter and Oleomargarine.—Theoretically the heats of combustion of butter fat and oleomargarine are different and de Schweinitz and Emery propose to utilize this difference for analytical purposes.²⁴ The samples of pure butter fat examined by them afforded 9,320, 9,327 and 9,362 calories, respectively. The calories obtained for various samples of oleomargarine varied from 9,574 to 9,795. On mixing butter fat and oleomargarine, a progressive increase in calorimetric power is found, corresponding to the percentage of the latter constituent. Lards examined at the same time gave from 9503 to 9654 calories.

FRUITS, MELONS AND VEGETABLES.

608. Preparation of Sample.—Fresh fruits and vegetables are most easily prepared for analysis by passing them through the pulping machine described on page 10. Preliminary to the pulp-

²⁴ Journal of the American Chemical Society, 1896, 18 : 174.

ing they should be separated into skins, cores, seeds and edible portions, and the respective weights of these bodies noted. Each part is separately reduced to a pulp and, at once, a small quantity of the well mixed substance placed in a flat bottom dish and dried in a vacuum oven below 70° , and the percentage of water contained in the sample determined. For the determination of the relative amounts of those constituents which are only present in small quantities and are not altered by heat, the bulk of the sample, three or four kilograms, is dried on a tray of tinned or aluminum wire, first at a low and then at a high temperature, until all or nearly all the moisture is driven off. The dried pulp is then ground to as fine a powder as possible and subjected to the ordinary processes of analysis; *viz.*, the determination of the moisture, ash, nitrogen, fiber, fat and carbohydrates.

In this method of analysis it is customary to determine the carbohydrates, exclusive of fiber, by subtracting the sum of the per cent. of the other constituents and the nitrogen multiplied by 6.25 from 100.

609. Examination of the Fresh Matter.—To avoid the changes which take place in drying fruits and vegetables, it is necessary to examine them in the fresh state. The samples may be first separated into meat and waste, as suggested above, or shredded as a whole. The moisture in the pulp is determined as indicated above, and in a separate portion the soluble matters are extracted by repeated treatment with cold water. The seeds, skins, cellulose, pectose and other insoluble bodies are thus separated from the sugars, pectins, pectic and other acids, and other soluble matters. The insoluble residue is rapidly dried and the relative proportions of soluble and insoluble matters determined. The estimation of these bodies is accomplished in the usual way.

610. Separation of the Carbohydrates.—It is often desirable to determine the relative proportions of the more important carbohydrates which are found in fruits and vegetables. The pentoses and pentosans are estimated by the method described in paragraph 194. The cane sugar, dextrose and levulose are determined by extracting a portion of the substance with eighty per cent.

alcohol and estimating the reducing sugars in the extract before and after inversion by the processes described in paragraphs 153-191. Water may be employed as the solvent in the ordinary fruits as they contain practically no gum. The percentages of sugars deducted from the percentage of carbohydrates, exclusive of fiber, give the quantity of gums, pentosans, cellulose and pectose bodies present.

Pectose exists chiefly in unripe fruits. By the action of the fruit acids and of a ferment, pectose, in the process of ripening, is changed into pectin and similar hydrolyzed bodies soluble in water. The gelatinous properties of boiled fruits and fruit juices are due to these bodies, boiling accelerating their formation. In very ripe fruits the pectin is completely transformed into pectic acids.²⁵

611. Examination of Fruit and Vegetable Juices.—The fruits and vegetables are pulped, placed in a press and the juices extracted. The pressure should be as strong as possible and the press described in paragraph 247 is well suited to this purpose. The specific gravity of the expressed juice is obtained and the sucrose therein determined by polarization before and after inversion. The reducing sugars and the relative proportions of dextrose and levulose are determined in the usual manner.

The majority of fruits contain both sucrose and invert sugar, the relative proportions depending on the variety and in some cases on the degree of maturity and the length of time and temperature of storage after the fruit is harvested. In some fruits as the persimmon and the majority of varieties of grape the sugar is all inverted. In apples sucrose increases till the fruit is ripe and then decreases owing to conversion into invert sugar. Soluble gums, dextrin, pectin, etc., may be separated from the sugars by careful precipitation with alcohol, or the total solids, ash, nitrogen, ether extract and acids be determined and the carbohydrates estimated by difference. From the carbohydrates the

²⁵ Fremy, *Journal de Pharmacie et de Chimie*, 1817 [3]; 12: 13-24.

Comptes rendus hebdomadaires de Seances de l'Academie de Sciences, 1847, 24: 1046.

total percentage of sugars is deducted and the remainder represents the quantity of pectin, gum and other similar bodies present.

612. Separation of Pectin.—Pectin exists in considerable quantities in the juice of ripe fruits (pears) and may be obtained in an approximately pure state from the juices by first removing proteins by the careful addition of tannin, throwing out the soluble lime salts with oxalic acid and precipitating the pectin with alcohol. The properties and varieties of pectin are but vaguely understood, there seems to be no satisfactory evidence of the existence of the meta and parapectins of the earlier writers. Bigelow and Gore have made a careful experimental study of the combustion of fruits, summarized the literature of the subject and given the most reliable data of their composition and of the changes which take place during storage and ripening.²⁶

Pectic acid may be obtained by boiling an aqueous extract (carrots) with sodium carbonate and precipitating the pectic with hydrochloric acid. It is a jelly-like body and dries to a horny mass.

613. Determination of Free Acid.—The free acid, or rather total acidity of fruits, is determined by the titration of their aqueous extracts or expressed juices with a set alkali. In common fruits and vegetables the acidity is calculated to malic $C_4H_8O_6$, in grapes to tartaric $C_4H_6O_6$, and in citrous fruits to citric acid $C_6H_8O_7$. Many other acids are found in fruits and vegetables, but those mentioned are predominant in the classes given.

614. Composition of Ash of Fruits.—Two or three kilograms of the dried sample are incinerated at a low temperature and

²⁶ Bureau of Chemistry, U. S. Department of Agriculture, Bulletins 66 (revised), 87, 94 and 97, 1904-1905.

Journal of the American Chemical Society, 1905, **27** : 915, and 1906, **28** : 688.

Tollens, (Liebig's) *Annalen der Chemie*, 1895, **286** : 278-295.

Goldthwaite, *Journal of Industrial and Engineering Chemistry*, 1909,

1 : 333.

Journal de Pharmacie et de chimie, 1899, [6] **9** : 163, 281 and 513.

burned to a white ash in accordance with the directions given in paragraphs 36-46.

The composition of the ash is determined by the methods already described.²⁷

The pure ash of some common whole fruits has the following composition:²⁸

Name	Per cent. pure ash in fruit	Per cent. potash	Per cent. soda	Per cent. lime	Per cent. magnesia	Per cent. ferric oxide	Per cent. manganese-manganic oxide	Per cent. phosphorous pentoxid	Per cent. sulphur trioxid	Per cent. silica	Per cent. chlorin
Prune	0.47	63.83	2.65	4.66	5.47	2.72	0.39	14.08	2.68	3.07	0.34
Apricot	0.51	59.36	10.26	3.17	3.68	1.68	0.37	13.09	2.63	5.23	0.45
Orange	0.43	48.94	2.50	22.71	5.34	0.97	0.37	12.37	5.25	0.65	0.92
Lemon	0.53	48.26	1.76	29.87	4.40	0.43	0.28	11.09	2.84	0.66	0.39
Apple	1.44	35.68	26.09	4.08	8.75	1.40	—	13.59	6.09	4.32	—
Pear	1.97	54.69	8.52	7.98	5.22	1.04	—	15.20	5.69	1.49	—
Peach	4.90	27.95	0.23	8.81	17.66	0.55	—	43.63	0.37	—	—

615. Dried Fruits.—A method of preserving fruits largely practiced consists in subjecting them, in thin slices or whole, to the action of hot air until the greater part of the moisture is driven off. The technique of the process is fully described in recent publications.²⁹ It has been shown by Richards that fruit subjected to rapid evaporation undergoes but little change aside from the loss of water.³⁰

Raisins, pears, and stone fruits are sun dried in California where the air is dry and hot and freedom from rain is assured during the drying season. Pears and stone fruits (except prunes) are first cut in halves, cores and pits removed and the

²⁷ This work, Vol. 2 (revised) : 618.

²⁸ California Agricultural Experiment Station, Bulletins 93, 97, 101 and 102, 1891-1893.

Wolf, Aschen Analyse, 1871, 1 : 126-127.

²⁹ Cornell Agricultural Experiment Station, Bulletin 100, 1895,

Chemical Division, U. S. Department of Agriculture, Bulletin 48, 1896 : 10.

³⁰ U. S. Department of Agriculture, Annual Report, 1886 : 353.

fruit treated with the fumes of burning sulfur till it becomes soft. Prunes are dried whole but the skin is first "checked" with a solution of hot caustic soda. Raisins are sometimes dried after checking the skins with a soap prepared from olive oil and a solution of caustic soda, and sometimes without preliminary treatment. The use of fumes of burning sulfur is objectionable from hygienic and other reasons. The use of lye and other chemicals should be limited to only such quantities as can subsequently be removed before consumption.

In the analyses of dried fruits the methods already described are used. The presence of pectin renders the filtration of the aqueous extract somewhat difficult, and in many cases it is advisable to determine the sugars present in the extract without previous filtration.

616. Zinc in Evaporated Fruits.—Fruits were formerly evaporated on trays made of galvanized iron. In these instances a portion of the zinc is dissolved by the fruit acids, and will be found as zinc malate, etc., in the finished product. The presence of zinc salts is objectionable for hygienic reasons, and therefore the employment of galvanized trays should be discontinued. The presence of zinc in evaporated fruits may be detected by the following process.³¹ The sample is placed in a large platinum dish and heated slowly until dry and in incipient combustion. The flame is removed and the combustion allowed to proceed, the lamp being applied from time to time in case the burning ceases. When the mass is burned out it will be found to consist of ash and char, which are ground to a fine powder and extracted with hydrochloric or nitric acid. The residual char is burned to a white ash at a low temperature, the ash extracted with acid, the soluble portion added to the first extract and the whole filtered. The iron in the filtrate is oxidized by boiling with bromin water and the boiling continued until the excess of bromin is removed. A drop of methyl orange is placed in the liquid and ammonia added until it is only faintly acid. The

³¹ Chemical Division, U. S. Department of Agriculture, Bulletin 48, 1896 : 26.

iron is precipitated by adding 50 cubic centimeters of a solution containing 250 grams of ammonium acetate in a liter and raising the temperature to about 80°. The precipitate is separated by filtration and washed with water at 80° until free of chlorids. The filtrate is saturated with hydrogen sulfid, allowed to stand until the zinc sulfid settles and poured on a close filter. It is often necessary to return the filtrate several times before it becomes limpid. The collected precipitate is washed with a saturated solution of hydrogen sulfid containing a little acetic acid. The precipitate and filter are transferred to a crucible, dried, ignited and the zinc weighed as oxid. Small quantities of zinc salts added to fresh apples which were dried and treated as above described, were recovered by this method without loss. Other methods of estimating zinc in dried fruits are given in the bulletin cited.

Evaporated apples contain a mean content of 23.85 per cent. of water and 0.931 per cent. of ash.

The mean quantity of zinc oxid found in samples of apples dried on zinc trays is 10 milligrams for each 100 grams of the fruit. When zinc exists in the soil it will be found as a natural constituent in the crop.³²

617. Composition of Watermelons and Muskmelons.—In the examination of melons a separation of the rind, seeds and meat is somewhat difficult of accomplishment, since the line of demarcation is not distinct. In watermelons the separation of rind and meat is made at the point where the red color of the meat disappears. In muskmelons no such definite point is found and in the examination of these they are taken as a whole. The total moisture, ash and nitrogen may be determined in the whole mass or in the separate portions. The sugars are most conveniently determined in the expressed juices. The mean composition of the melons given below is that obtained from analyses made in the Department of Agriculture.³³

³² Arkansas Agricultural Experiment Station, Bulletin 42, 1896 : 78.

³³ U. S. Department of Agriculture, Annual Report, 1886 : 347.

COMPOSITION OF MELONS.

	Total weight, grams.	Juice, per cent.	Total sugars, per cent.	Ash, per cent.
Watermelons...	10,330	{ meat 83.99 rind 81.02	6.12	0.37
Muskmelons...	3,407	80.23	6.45	0.57

COMPOSITION^s OF JUICE.

	Sucrose in juice, per cent.	Reducing sugars in juice, per cent.	Ash in juice, per cent.
Watermelons...	{ meat 1.92 rind 0.34	{ meat 4.33 rind 2.47	{ meat 0.31 rind 0.33
Muskmelons...	1.02	3.04	0.53

Later and extensive investigations made by Straughn, Church and Bryan show wide variations in the sugar content of cantaloupes.³⁴

The sugar in the juice from the rinds and from the meats was found in the quantities given below:

	Sucrose, per cent.	Invert sugar, per cent.
Rinds	{ maximum 3.99 minimum 0.23	6.33 1.86
Meats	{ maximum 6.61 minimum 0.94	4.28 2.05

TEA AND COFFEE.

618. Special Analysis.—Aside from the examination of teas and coffees for adulterants, the only special determinations which are required in analyses are the estimation of the alkaloid (cafein) and of the tannin contained therein. It is chiefly to the alkaloid that the stimulating effects of the beverages made from tea and coffee are due. The determination of the quantity of tannin contained in tea and coffee is accomplished by the processes described under the chapter devoted to that glucosid.

The general analysis, *viz.*, the estimation of water, ether extract, total nitrogen, fiber, carbohydrates and ash, with the exceptions noted above, is conducted by the methods which have already been given.

For detailed instructions concerning the detection of adulter-

³⁴ Unpublished data.

ants of tea and coffee the bulletins of the Bureau of Chemistry, Department of Agriculture, may be consulted.³⁵

619. Estimation of Caffein (Thein).—The method adopted by Spencer, after a thorough trial of all the usual processes for estimating this alkaloid, is as follows:³⁶ To three grams of the finely powdered tea or coffee, in a 300 cubic centimeter flask, add about a quarter of a liter of water, slowly heat to the boiling point, using a fragment of tallow to prevent frothing, and boil gently for half an hour. When boiling begins, the flask should be nearly filled with hot water and more added from time to time to compensate for the loss due to evaporation. After cooling, add a strong solution of basic lead acetate until no further precipitation is produced, complete the volume to the mark with water, mix and throw on a filter. Precipitate the lead from 50 cubic centimeters of the filtrate by hydrogen sulfid filter and wash the precipitate with hot water. Boil the filtrate to expel the excess of hydrogen sulfid, transfer 50 cubic centimeters of this solution to a separatory funnel and shake seven times with chloroform solution in a tared flask and remove the solvent by gentle distillation. A safety bulb, such as is used in the kjeldahl nitrogen method, should be employed to prevent entrainment of caffein with the chloroform vapors.

The extraction with chloroform is nearly complete after shaking out four times; a delicate test, however, will usually reveal the presence of caffein in the watery residue even after five or six extractions, hence seven extractions are recommended for precautionary reasons. The residual caffein is dried at 75° for two hours and weighed.

The principal objection which has been made to Spencer's method is that the boiling with water is not continued for a suf-

³⁵ Chemical Division, U. S. Department of Agriculture, Bulletin 13, 1892 : 875 et seq.

Bureau of Chemistry, U. S. Department of Agriculture, Bulletin 107 (revised), 1912 : 149 and 152.

³⁶ Journal of Analytical and Applied Chemistry, 1890, 4 : 391.

Chemical Division, U. S. Department of Agriculture, Bulletin 13, 1892 : 889.

ficient length of time. For the water extraction, Allen prescribes at least six hours cohobation.³⁷ In this method six grams of the powdered substance are boiled with half a liter of water for six hours in a flask, with a condenser, the decoction filtered, the volume of the filtrate completed to 600 cubic centimeters with the wash water, heated to boiling, and four cubic centimeters of strong lead acetate solution added, the mixture boiled for 10 minutes, filtered and half a liter of the filtrate evaporated to 50 cubic centimeters. The excess of lead is removed with sodium phosphate and the filtrate and washings concentrated to about 40 cubic centimeters. The caffeine is removed by shaking four times with chloroform. Older but less desirable processes are fully described by Allen.³⁸

In France this method is known as the process of Petit and Legrip, and it has been worked out in great detail by Grandval and Lajoux and by Petit and Terbat.³⁹

620. Estimation of Caffeine by Precipitation with Iodin.—The caffeine in this method is extracted, the extract clarified by lead acetate and the excess of lead removed as in Spencer's process described above. The caffeine is determined in the acidified aqueous solution thus prepared, according to the plan proposed by Gomberg, as follows:⁴⁰

Definite volumes of the aqueous solution of the caffeine are acidulated with sulfuric and the alkaloid precipitated by an excess of a set solution of iodine in potassium iodide. After filtering, the excess of iodine in an aliquot part of the filtrate is determined by titration with a tenth normal solution of sodium thiosulfate. The filtration of the iodine liquor is accomplished on glass wool or asbestos. The results of the analyses are calculated from the composition of the precipitated caffeine periodide; *viz.*, $C_8H_{10}N_4O_2I_4$. The weight of the alkaloid is calculated from the amount of iodine required for the precipitation by the equation

³⁷ Pharmaceutical Journal, 1892-93, 3rd ser. 23 : 217.

³⁸ Allen, Commercial Organic Analysis, 1892, 2d ed. (revised and enlarged), 3 : pt. 2, 484.

³⁹ Journal de Pharmacie et de Chimie, 1896 (6) 3 : 529-534.

⁴⁰ Journal of the American Chemical Society, 1896, 18 : 338.

$4\text{I}:\text{C}_8\text{H}_{10}\text{N}_4\text{O}_2 = 508:194$. From this equation it is shown that one part of iodine is equivalent to 0.3819 part of caffeine, or one cubic centimeter of tenth normal iodine solution is equal to 0.00485 gram of iodine.

In practice, it is recommended to divide the aqueous extract of the alkaloid, prepared as directed above, into two portions, one of which is treated with the iodine reagent without further preparation, and the other after acidulation with sulfuric. After 10 minutes, the residual iodine is estimated in each of the solutions as indicated above. The one portion, containing only the acetic acid resulting from the decomposition of the lead acetate, serves to indicate whether the aqueous solution of the caffeine contains other bodies than that alkaloid capable of forming a precipitate with the reagent, since the caffeine itself is not precipitated even in presence of strong acetic acid.

In the solution acidulated with sulfuric, the caffeine, together with the other bodies capable of combining with iodine, is precipitated. The residual iodine is determined in each case, and thus the quantity which is united with the caffeine is easily ascertained. The weight of iodine which has entered into the precipitated caffeine periodide multiplied by 0.3819 gives the weight of the caffeine in the solution.

Gomberg's method has been subjected to a careful comparative study by Spencer and has been much improved by him in important particulars.⁴¹

It is especially necessary to secure the complete expulsion of the hydrogen sulfide and to observe certain precautions in the addition of the iodine reagent. The precipitation should be made in a glass-stoppered flask, shaking thoroughly after the addition of the iodine and collecting the precipitate on a gooch. As thus modified, the iodine process gives results comparable with those obtained by Spencer's method, and it can also be used to advantage in estimating caffeine in headache tablets in the presence of acetanilide.

621. Freeing Caffeine of Chlorophyll.—Any chlorophyll which

⁴¹ Manuscript communication to author.

may pass into solution and be found in the caffeine may be removed by dissolving the caffeine in 10 per cent. sulfuric acid, filtering, neutralizing with ammonia and evaporating to dryness. The residue is taken up with chloroform, the chloroform removed at a low temperature and the pure caffeine thus obtained.⁴²

622. Protein Nitrogen.—The protein nitrogen in tea and coffee may be determined in the residue after extraction of the alkaloid by boiling water as described above. More easily it is secured by determining the total nitrogen in the sample and deducting therefrom the nitrogen present as caffeine. The remainder, multiplied by 6.25, will give the quantity of protein matter.

623. Carbohydrates of the Coffee Bean.—The carbohydrates of the coffee bean include those common to vegetable substances; *viz.*, cellulose, pentosan bodies (xylan, araban), fiber, etc., together with certain sugars, of which sucrose is pointed out by Ewell as the chief.⁴³ In smaller quantities are found a galactose yielding body (galactan), as pointed out by Maxwell, a dextrinoid and a trace of a sugar reducing alkaline copper solution.

The sucrose may be separated from the coffee bean by the following process:⁴⁴ The finely ground flour is extracted with 70 per cent. alcohol, the extract clarified with lead acetate, filtered, the lead removed from the filtrate with hydrogen sulfid, the excess of the gas removed by boiling, the filtrate evaporated in a partial vacuum to a sirup and the sucrose crystallized from a solution of the sirup in alcohol.

For a quantitative determination, 10 grams of the coffee flour are extracted with ether and the residue with 75 per cent. alcohol. This process, conducted in a continuous extraction apparatus, should be continued for at least 24 hours. The alcohol is removed by evaporation, the residue dissolved in water, clarified with basic lead acetate, filtered, the precipitate washed, the lead removed, again filtered, the filtrate washed and wash water and filtrate made to a definite volume. In an aliquot part of this

⁴² Journal de Pharmacie et de Chimie, 1896 (6), 3 : 533.

⁴³ American Chemical Journal, 1892, 14 : 473-476.

⁴⁴ American Chemical Journal, 1892, 14 : 474.

solution the sugars are determined by the alkaline copper method, both before and after inversion. From the data obtained the percentage of sucrose is calculated.

In a coffee examined by Ewell the percentage of sucrose was found to be 6.34. The pentose yielding constituents of the coffee bean amount to from eight to 10 per cent.

When coffee meal is extracted with a five per cent. solution of sodium carbonate, a gummy substance is obtained, which is precipitable by alcohol. This gum, after washing with hydrochloric acid containing alcohol, gives a gray, translucent, hard mass on drying. On hydrolysis it yielded 75.2 per cent. of dextrose, on distillation with hydrochloric acid, 13 per cent. of furfuraldehyd and, on oxidation with nitric acid, 18.7 per cent. of mucic acid. This gum, therefore, consists chiefly of a mixture of galactan, xylan and araban.

The method of procedure preferred for the estimation of the pentosans is that described in paragraph 194. The phloroglucin is dissolved in hydrochloric acid of 1.06 specific gravity before it is added to the furfural distillate. The latest factor for converting the phloroglucin obtained into furfural is to divide by 1.82 for small quantities and 1.93 for large quantities. After the furfural is obtained, the factors given above are applied.

624. Application of Roentgen Rays to Analysis.—The detection of mineral matters in vegetable substances by Roentgen photography has been proposed by Ranvez.⁴⁵ This process will prove extremely valuable in detecting the facing of teas with mineral substances. Practically, it has been applied by Ranvez in the detection of mineral substances mixed with saffron with fraudulent intent.

Barium sulfate is often mixed with saffron for the purpose of increasing its weight. Pure saffron and adulterated samples are enclosed in capsules of black paper and exposed on the same sensitive plate for a definite time to the rays emanating from a Crookes tube. In this case the pure saffron forms only a very faint shadow in the developed negative, while the parts to which

⁴⁵ *Comptes rendus hebdomadaires de Scances de l'Academie de Sciences*, 1896, **122** : 841.

barium sulfate are attached produce strong shadows. The principle involved is applicable to a wide range of analytical research.

625. Read Method of Detecting Colored Teas.^{45a}—Place two ounces of tea in a sieve five inches in diameter, having 40 meshes to the inch, and sift a small quantity of the dust onto a semi-glazed white paper about eight by 10 inches. The amount of dust placed on the paper should be approximately the weight of one-eighth of a silver half dime, or about two grains. To get the requisite amount of dust it is sometimes necessary to rub the leaf against the bottom of the sieve. The dust should be well distributed or peppered over the surface of the paper. The paper is placed on a plain, firm surface, preferably glass or marble, and the dust crushed by drawing over it, with considerable pressure, a flat steel spatula about five inches long. This is done repeatedly, the tea dust being ground almost to a powder and the particles of coloring matter, if any, being thus spread or streaked on the paper, so as to become more apparent. The loose dust may then be blown off, and the paper examined by means of a simple lens magnifying seven and one-half diameters. In distinguishing these particles and streaks bright light is essential.

The crushed leaf in either black or green tea appears in such quantity that there is no chance of mistaking the leaf for coloring or facing material.

This test is performed in comparison with the standard, and if the tea is clearly equal to the standard as regards coloring or facing matter, the operation need not be repeated. If particles of coloring or facing are found in the sample under comparison with the standard, this operation should be repeated a sufficient number of times for the examiner to satisfy himself as to whether or not the tea is in fact equal thereto. If found not equal to the standard, samples should be drawn from packages representing at least five per cent. of the line in question and subjected to the above test, and if a majority of these samples are below the standard, a test sheet of the tea in question should then be sent to the local appraiser's chemist or to the

^{45a} Treasury Department, T. D. 33211, 1913 : 9.

nearest pure-food laboratory of the Department of Agriculture for identification of the coloring or facing matter disclosed. As soon as the coloring or facing matter is identified, then the tea should be rejected.

The above method was devised by Miss Read of the Bureau of Chemistry and is the most delicate and reliable method of detecting the coloring of teas that has yet been discovered. During the fiscal year ending June 30th, 1913, 513,633 pounds of tea were refused admission to the United States by reason of being colored.

TANNINS AND ALLIED BODIES.

626. Occurrence and Composition.—The tannins and allied bodies, which are of importance in this connection, are those which occur in food products and beverages and also those made use of in the leather industry. The term tannin is applied to a large class of astringent substances, many of which are glucosids. Tannic acid is the chief constituent of the tannins, and is found in a state of comparative purity in nutgalls. The source from which the tannic acid is derived is indicated by a prefix to the name, *e. g.*, gallotannic, from nutgalls, and caffetannic, from coffee, etc. The tannins have lately been the theme of a critical study by Trimble, and the reader is referred to his work for an exhaustive study of the subject.⁴⁶ Tannin is one of the most widely diffused compounds, occurring in hundreds of plants. Commercially, the oaks and hemlocks are the most important plants containing tannin. The sumac, mangrove, canaigre, palmetto and many others have also been utilized as commercial sources of tannin. The tannins as a class are amorphous and odorless. They are slightly acid and strongly astringent. Their colors vary from dark brown to pure white. They are soluble in water, alcohol, ether and glycerol and insoluble in chloroform, benzene, petroleum ether, carbon bisulfid and the oils. The tannine give a blue color with ferric Salts. They are all precipitated by gelatin or albumin. Tannins are not only generally of a

⁴⁶ Trimble, *The Tannins*, 1892-1894 : 1-2.

glucosidal nature, but are found quite constantly associated with reducing sugars, or in unstable combination therewith.

The reducing sugars may be separated from the tannin by precipitating the latter with lead acetate and determining the glucose in the filtrate after the removal of the lead. A separate portion of the tannin is hydrolyzed with sulfuric or hydrochloric acid and the reducing sugars again determined. Any excess of sugars over the first determination is due to the hydrolysis of the tannin glucosid.

627. Detection and Estimation of Tannins.—The qualitative reactions above mentioned serve to detect the presence of a tannin. Of the iron salts ferric acetate or chlorid is preferred. Ferrous salts do not give any reaction with dilute tannin solutions. An ammoniacal solution of potassium ferricyanid forms with tannins a deep red color changing to brown. In quantitative work the tannins are mostly determined by precipitation with metallic salts, by treatment with gelatin or hide powder, or by oxidation with potassium permanganate. Directions for the estimation of glucosids in general are found in Dragendorff's book.⁴⁷

628. Precipitation with Metallic Salts.—The methods depending on precipitation of the tannins with metallic salts are but little used and only one of them will be mentioned here. A full description of the others is contained in Trimble's book.⁴⁸ A method for the determination of caffetannic acid in coffee has been worked out by Krug and used with some satisfaction.⁴⁹

In this method two grams of the coffee meal are digested for 36 hours with 10 cubic centimeters of water, 25 cubic centimeters of 90 per cent. alcohol added and the digestion continued for 24 hours. The contents of the flask are poured on a filter and the residue washed with 90 per cent. alcohol. The filtrate contains tannin, caffetannic acid and traces of coloring matter and fat. It is heated to the boiling point and clarified with a solution of lead acetate. A caffetannate of lead containing 49 per cent. of

⁴⁷ Dragendorff, *Plant Analysis*, 1909: 50 et seq. Transl. by H. G. Greenish.

⁴⁸ Trimble, *The Tannins*, 1892, 1: 33.

⁴⁹ Chemical Division, U. S. Department of Agriculture, *Bulletin* 13, 1892: 908.

the metal is precipitated. As soon as the precipitate has become flocculent it is collected on a filter, washed with 90 per cent. alcohol until the soluble lead salts are all removed, then with ether and dried. The composition of the precipitate is represented by the formula $\text{Pb}_3(\text{C}_{15}\text{H}_{15}\text{O}_8)_2$. The caffetannic acid is calculated by the equation: Weight of precipitate: weight of caffetannic acid = 1,267:652.

629. The Gelatin Method.—The precipitation of tannin with gelatin is the basis of a process for its quantitative estimation which, according to Trimble, is conducted as follows:⁵⁰ Two and a half grams of gelatin and 10 grams of alum are dissolved in water and the volume of the solution made up to one liter. The solution of gelatin and also that of the tannin are heated to 70° and the tannin is precipitated by adding the gelatin reagent slowly, with constant stirring, until the precipitate coagulates, and, on settling, leaves a clear liquor in which no further precipitate is produced on adding a few drops more of the reagent. In case the clearing of the mixture does not take place readily, the process should be repeated with a more dilute tannin solution. The precipitate is collected on two counterpoised filter papers one placed inside the other, dried at 110° and weighed, the empty filter paper being placed on the pan with the weights. For pure tannin (gallotannic acid) 54 per cent. of the weight of the precipitate are tannin. Ammonium chlorid and common salt have been used in place of the alum in preparing the reagent, but if the proportion of alum mentioned above be used, satisfactory results will be obtained in most cases.

630. The Hide Powder Method.—The principle of this method is based on the change in specific gravity, *i. e.*, total solids, which a tannin solution will undergo when brought into contact with raw hides in a state of fine subdivision. The hide powder absorbs the tannin, and the total solid content of the solution is correspondingly diminished. The method is conducted according to the official directions as follows:⁵¹

⁵⁰ Trimble, *The Tannins*, 1892, 1 : 38.

⁵¹ Bureau of Chemistry, U. S. Department of Agriculture, Bulletin 107. Revised 1912 : 35.

1. *Crude Materials*.—(a) *Moisture in Sample as Received*.—Grind promptly and dry 10 grams as directed under “4. Evaporation and Drying.”

(b) *Preparation for Extraction*.—Dry the sample not above 60° and grind to pass through a 20-mesh sieve.

(c) *Moisture in Prepared Sample*.—Dry 10 grams as directed under “4. Evaporation and Drying,” and calculate all subsequent determinations on the moisture-free basis.

(d) *Extraction*.—Extract such a quantity of material as will give between 0.35 and 0.45 gram of tannin per 100 cubic centimeters of solution in an extractor which permits the removal of the extract solution without allowing it to boil, using a layer of cotton to prevent fine material from passing over. Collect from 400 to 500 cubic centimeters in this way, remove and continue extraction with a fresh portion of water at steam heat in the usual way until the material is free of tannin, testing the last few cubic centimeters of extract with gelatin-salt solution.

For spent materials approximate the above proportions as closely as practicable.

(e) *Analysis*.—Heat the extract to 80° and proceed as directed under extracts. In case weaker solutions than the method specifies are employed the amount of hide powder used must be reduced in proportion to the quantity of tannin present.

2. *Extracts*.—(a) *Preparation of Solution*.—Grind solid extracts rapidly, and thoroughly mix. Heat fluid extracts to 50°, thoroughly mix, cool to room temperature, and weigh from weighing bottles. Dissolve in 900 cubic centimeters of water at 80° such a quantity of the extract as will give from 0.35 to 0.45 gram of tannin in 100 cubic centimeters of solution. Allow to cool slowly for from 12 to 20 hours at a temperature not below 20° and dilute to one liter.

(b) *Total Solids*.—Thoroughly mix the solution, immediately pipette 100 cubic centimeters into a tared dish, evaporate, and dry as directed under “4. Evaporation and Drying.”

(c) *Soluble Solids*.—Add 75 cubic centimeters of solution (kept at from 20° to 25° during filtration) to two grams of kaolin (free from soluble salts), stir, let stand 15 minutes, decant.

and discard as much as possible of the supernatant liquid, and again add 75 cubic centimeters of the tannin solution to the kaolin. Stir, and pour immediately on a 15 centimeter, No. 590 folded filter. Keep the filter full and the funnel and receiving vessel covered. Reject the first 150 cubic centimeters of filtrate, evaporate and dry the next 100 cubic centimeters (which must be as clear as practicable), as directed under "4. Evaporation and Drying."

(d) *Nontannins*.—Prepare a sufficient quantity of hide powder in the following manner: Digest with 25 times its weight of water until thoroughly soaked; add three per cent. of chrome alum in solution, agitate occasionally for several hours, and allow to stand over night. Wash, by squeezing through linen, until the wash water gives no precipitate with barium chlorid. Squeeze the hide, using a press if necessary, so that it contains from 70 to 75 per cent. of water, and determine moisture (20 grams is a convenient quantity).

Add to 200 cubic centimeters of the tannin solution such a quantity of the wet hide as contains from 12 to 13 grams of dry hide, shake for 10 minutes in a shaker and squeeze immediately through linen. Add two grams of kaolin to the filtrate, stir, and filter through a folded 20 centimeter filter (No. 1 F Swedish recommended), returning until clear. Evaporate and dry 100 cubic centimeters as directed under "4. Evaporation and Drying." Correct the weight of the residue for dilution caused by the water contained in the wet hide powder.

The nontannin filtrate must not give a precipitate with a gelatin-salt solution (one per cent. of gelatin and 10 per cent. of salt).

(e) *Tannin*.—The difference between the weight of the soluble solids and the corrected nontannin residue is the weight of tannin in 100 cubic centimeters of solution.

3. *Liquors*.—(a) *Preparation of Solution*.—Dilute to contain approximately 0.7 gram of solids in 100 cubic centimeters of solution.

(b) *Total Solids*.—Proceed as in extracts, 2 (b).

(c) *Soluble Solids*.—Proceed as in extracts, 2 (c).

(d) *Nontannins*.—Shake 200 cubic centimeters of solution as directed under extracts with such an amount of the wet chromed hide powder (2 (d)) as will give the following proportions:

Dry hide.	Tannin in 200 cubic centimeters.
8 to 10 grams	0.7 to 0.8 gram.
5 to 8 "	0.5 to 0.7 "
2 to 5 "	0.3 to 0.5 "
0 to 2 "	0. to 0.3 "

Evaporate and dry 100 cubic centimeters as directed under "4. Evaporation and Drying."

4. *Evaporation and Drying*.—Evaporate and dry for 16 hours in a combined evaporator and dryer at from 98° to 100°; or after evaporating, dry for 12 hours on the bottom shelf of a water oven at from 98° to 100°. Conduct evaporation and drying in flat-bottom glass dishes from two and three-quarters to three inches in diameter.

5. *Acidity of Liquors*.—Dilute 100 cubic centimeters of liquor to 500 cubic centimeters. Add two grams of purified animal charcoal to 100 cubic centimeters of the dilute liquor in a flask provided with a tube condenser. Heat to boiling with frequent shaking, cool, filter, and titrate an aliquot with tenth-normal alkali.

631. The Permanganate Gelatin Method.—This process, which is essentially the method of Löwenthal, as improved by Counciler, Schroeder and Procter and as used by Spencer for the determination of tannin in teas, is conducted as described below.⁵² The principle of the process is based on the oxidation of all bodies in solution oxidizable by potassium permanganate, the subsequent precipitation of the tannin by a gelatin solution, and the final oxidation, by means of permanganate, of the remaining organic

⁵² Bureau of Chemistry, U. S. Department of Agriculture, Bulletin 107 (revised), 1912 : 150-151.

Zeitschrift für analytische chemie, 1886, 25 : 122.

Journal of the Society of Chemical Industry, 1884, 3 : 82.

Trimble, The Tannins, 1892, 1 : 43-44.

bodies. The difference between the total oxidizable matter and that left after the precipitation of the tannin represents the tannin originally in solution.

Reagents Required.—The following reagents are necessary to the proper conduct of the potassium permanganate process:

(1) Potassium permanganate solution containing about one and a third grams of the salt in a liter:

The potassium permanganate solution is set by titration against the decinormal oxalic acid solution mentioned below. The end reaction with the indicator must be of the same tint in all the titrations, i. e., either golden yellow or pink.

(2) Tenth-normal oxalic acid solution for determining the exact titer of the permanganate solution: (6.3 grams per liter.)

(3) Indigo-carmin solution to be used as an indicator and containing six grams of indigo-carmin and 50 cubic centimeters of concentrated sulfuric acid in a liter. The indigo-carmin must be very pure and quite free of indigo-blue.

(4) Gelatin solution, prepared by digesting 25 grams of gelatin at room temperature for one hour in a saturated solution of sodium chlorid, then heating until solution is complete, cooling and making the volume up to one liter:

(5) A salt acid solution, made by adding to 975 cubic centimeters of a saturated solution of sodium chlorid, enough strong sulfuric acid to bring the volume of the mixture to one liter:

(6) Powdered kaolin for promoting filtration.

The Process.—Five grams of the finely powdered tea (or other vegetable substance containing tannin) are boiled with 400 cubic centimeters of distilled water for half an hour. The distilled water should be at room temperature when poured over the powdered tea. After cooling, the volume of the decoction is completed to half a liter, and the contents of the flask poured on a filter. To 10 cubic centimeters of the filtered tea infusion are added two and a half times as much of the indigo-carmin solution and about three-quarters of a liter of distilled water.

The permanganate solution is run in from a burette, a little at a time, with vigorous stirring, until the color changes to a

light green, and then drop by drop until the final color selected for the end of the reaction, golden yellow or faint pink, is obtained. The number of cubic centimeters of permanganate required is noted and represented by a in the formula below. The titration should be made in triplicate and the mean of the two more nearly agreeing readings taken as the correct one.

One hundred cubic centimeters of the filtered tea infusion, obtained as directed above, are mixed with half that quantity of the gelatin reagent, the first named quantity of the acid salt solution added, together with 10 grams of the powdered kaolin, the mixture well shaken for several minutes and poured on a filter. Twenty-five cubic centimeters of the filtrate, corresponding to 10 of the original tea solution are treated with indigo solution and titrated with the permanganate reagent, under the conditions given above, and the reading of the burette made and represented by b . The quantity of permanganate solution, *viz.*, c , required to oxidize the tannin is calculated from the formula $a-b=c$. The relation between the permanganate, oxalic acid and tannin is such that 0.04157 gram of gallotannic acid is equivalent to 0.063 gram of oxalic acid. The relation between the oxalic acid solution and the permanganate having been previously determined the data for calculating the quantity of tannin, estimated as gallotannic acid, are at hand.

632. The Permanganate Hide Powder Method.—Instead of throwing out the tannin with gelatin it may be absorbed by hide powder. The principle of the process, save this modification, is the same as in the method just described. As described by Trimble, the analysis is conducted according to the following directions:⁵³

Reagents Required.—The reagents required for conducting the permanganate hide powder process are as follows:

1. *Permanganate Solution.*—Ten grams of pure potassium permanganate are dissolved in six liters of water. The solution is standardized with pure tannin. The moisture in the pure tannin is determined by drying at 100° to constant weight and then a

⁵³ Trimble, *The Tannins*, 1892, 1 : 48.

quantity of the undried substance, representing two grams of the dried material, is dissolved in one liter of water. Ten cubic centimeters of this solution and double that quantity of the indigo solution to be described below, are mixed with three-quarters of a liter of water and the permanganate solution added from a burette with constant stirring until the liquid assumes a greenish color and then, drop by drop, until a pure yellow color with a pinkish rim is obtained. Fifty cubic centimeters of the pure tannin solution are digested, with frequent shaking, with three grams of hide powder which has been previously well moistened and dried in a press for 18 or 20 hours, the contents of the flask thrown on a filter and 10 cubic centimeters of the filtrate titrated with the permanganate solution as directed above. The difference between the amount of permanganate solution required for the first and second titrations represents the amount of pure tannin or oxidizable matter removed by the hide powder.

2. *Indigo Solution.*—The indicator which is used in the titrations is prepared by dissolving 30 grams of sodium sulfindigotate in three liters of dilute sulfuric acid made by adding one volume of the strong acid to three volumes of water. The solution is shaken for a few minutes, thrown upon a filter and the insoluble residue washed with sufficient water to make the volume of the filtrate six liters.

3. *Hide Powder.*—The hide powder used should be white, wooly in character and sufficiently well extracted with water to afford no other extract capable of oxidizing the permanganate solution.

The Process.—The reagents having been prepared and tested as above, the solution of the substance containing the tannin, prepared as described further on, is titrated first with the permanganate solution in the manner already given. Fifty cubic centimeters of the tannin solution are then shaken, from time to time for 18 hours, with three grams of hide powder, thrown upon a filter and 10 cubic centimeters of the filtrate titrated with the potassium permanganate as above described. From the data obtained, the quantity of permanganate solution corresponding to

the tannin removed by the hide powder is easily calculated. The value of the permanganate solution having been previously set with a pure tannin, renders easy of calculation the corresponding amount of pure tannin in the solution under examination.

633. Preparation of the Tannin Infusion.—A sample weighing about a kilogram should be secured, representing as nearly as possible the whole of the materials containing tannin in a given lot. The sample is reduced to a fine powder and passed through a sieve containing apertures about a millimeter in diameter. The quantity of the sample used for the extraction depends largely upon its content of tannin. Five grams of nutgalls, 10 grams of sumac or 20 grams of oak bark represent about the quantities necessary for these classes of tannin-holding materials. The sample is boiled for half an hour with half a liter of water, filtered through a linen bag into a liter flask and washed and pressed with enough water to make the volume of the filtrate equal to one liter. The proper quantities of this solution are used for the analytical processes described above.

634. Estimation with Silk.—Silk free from gum absorbs tannin readily and completely from solutions of tanning materials at 50°, but does not absorb gallic acid, glucose, etc. About five grams of silk is necessary for 0.1 gram of tannin dissolved in 100 cubic centimeters of water. The tannin may be estimated by (*a*) the increase in the weight of the silk, (*b*) the difference in the proportion of solid matter in the solution before and after the treatment with silk, (*c*) by titration with permanganate. The last method is the most exact, but *b* is simple and expeditious, and gives results sufficiently exact for practical purposes. The silk is prepared by boiling 20 grams of raw silk for 30 minutes with a solution of 50 grams of white soap dissolved in 500 cubic centimeters of water, this treatment being repeated and the silk then well washed. The tannin solution is prepared by treating in the usual way from 10 to 20 grams of the tanning material. One thousand cubic centimeters of the solution should contain about three grams of tannin. Twenty-five cubic centimeters of this solution is diluted to 250 cubic centimeters and

100 cubic centimeters of the diluted liquid is heated at 50° for five hours, but not more, with five grams of the prepared silk. Titration with permanganate and indigo, before and after the treatment with silk, is conducted in the usual way. The results with sumac and oak galls are practically identical with those obtained by precipitating the tannin with cat-gut or ammoniacal zinc acetate, and the method is more expeditious. The special reagent (silk) is very readily prepared and is practically constant in composition.⁵⁴

635. General Properties of Tannins.—Ferric chlorid is a general reagent for tannins. Certain groups of tannins which are derived from two or more molecules of protocatechuic acid by elimination of water are also precipitated by gelatin, albumin, alkaloids, potassium-antimony tartrate, and other compounds. The reactions of these substances and others with gallic, digallic, trigallic, ellagic, ellagitannic, and chebulic acids, and also with other bodies related to tannin are important. The reactions and decompositions which tannins undergo in the plants containing them and particularly the formation of phlobaphenes and "reds" are used, to some extent, as a basis for classification. The 'reds' are the red to reddish-brown coloring matters of the bark, which are formed in the same way as gallotannic acid by a process of dehydration of aromatic hydroxy-acids. The phlobaphenes and reds are both precipitated by gelatin and may be obtained from the respective tannins or tannogens by boiling with dilute sulfuric acid.⁵⁵

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636. Fermented and Unfermented Tobacco.—Samples of tobacco may reach the analyst either in the fermented or unfermented state. As a basis for comparison, it is advisable in all cases to determine the constituents of the sample before fermentation sets in. The analysis, after fermentation is complete, will then

⁵⁴ Vignon, (*Comptes rendus*, 1898, **127** : 369-372).

Journal Chemical Society, Abstracts, 1899, ii, **76** : 135.

⁵⁵ Kunz-Krause (*Chemisches Centralblatt*, 1899, **70** : Bd. 1, 559).

Journal of the Chemical Society, Abstracts, 1899, **76** : pt. 1, 762.

show the changes of a chemical nature which it has undergone during the process of curing and sweating. Only tobacco which has undergone fermentation is found to be in a suitable condition for consumption. In addition to the natural constituents of tobacco, it may contain, in the manufactured state, flavoring ingredients such as licorice and sugar, coloring matters and in some instances, it is said, opium or other stimulating drugs. It is believed, however, that opium is not found in manufactured tobacco in this country.

In researches made at the Connecticut Station it is shown that fermentation produces but little change in the relative quantities of nitric acid, ammonia, fiber and starch in the leaves, while those of nicotin, albuminoids and amids are diminished. This is not in harmony with the generally accepted theory that starch is inverted and fermented during the process.⁵⁶

The nature of the ferments which are active in producing the changes which tobacco undergoes in curing, is not definitely understood, but they are probably enzymes. Some of the organic constituents of the tobacco undergo a considerable change during the process. Any sugar which is found in the freshly cured leaves disappears wholly or in part. As products of fermentation may also be found succinic, fumaric, formic, acetic, propionic and butyric acids.

637. Acid and Basic Constituents of Tobacco.—In unfermented and fermented tobacco are found certain organic acids, among the most important of which are citric, malic, oxalic, and tannic. Of the inorganic acids the chief which are found are nitric, sulfuric and hydrochloric. Among the bases ammonia and nicotin are the most important. Ammonia is found in the unfermented tobacco in only small quantities, but in the fermented product it may sometimes reach as high as half a per cent. The presence of these two nitrogenous bases in tobacco renders the estimation of the protein matter contained therein somewhat tedious and difficult.

⁵⁶ Connecticut Agricultural Experiment Station (New Haven), Annual Report, 1892 : 30.

638. Composition of Tobacco Ash.—The mineral constituents of tobacco are highly important from a commercial point of view. The burning properties of tobacco depend largely upon the nature of its mineral constituents. A sample containing a large quantity of chlorids burns much less freely than one in which the sulfates and nitrates predominate. For this reason, the use of potash fertilizers containing large amounts of chlorin is injudicious in tobacco culture, the carbonates and sulfates of potash being preferred. The leaves of the tobacco plant contain a much larger percentage of mineral constituents than the stems, their respective contents of pure ash, that is ash free from carbon dioxid, carbon and sand, being about 17 and 8. The pure ash of the leaves has the following mean composition: Potash 29.1 per cent., soda 3.2 per cent, lime 36.0 per cent., magnesia 7.4 per cent, iron oxid 2.0 per cent, phosphoric acid 4.7 per cent., sulfuric acid 6.0 per cent., silica 5.8 per cent., and chlorin 6.7 per cent.⁵⁷

To obtain the best burning qualities, the potassium content must be five per cent. at least, and that of chlorin not over 0.6 per cent. Tobacco should be low in phosphoric acid as those high in this substance give a dark ash, presumably because the low melting phosphates by occlusion prevent complete ignition of carbonaceous materials.

The presence of lime and of citric and other organic acids seems to have a very favorable effect on the burning qualities of tobacco. The more mineral constituents tobacco has the higher its potassium content and the smaller its content of inorganic acids and the better its burning qualities. Potassium salts of organic acids are especially favorable.

639. Composition of Tobacco.—The mean composition of some of the more important varieties of water free tobacco is shown in the following table:⁵⁸

⁵⁷ Kissling, *Tabakkunde*, 1893, *Der Tabak im Lichte der neusten naturwissenschaftlichen Forschungen*, : 40.

⁵⁸ Kissling, *Tabakkunde*, : 58.

	Havana, per cent.	Sumatra, per cent.	Kentucky, per cent.	Java, per cent.
Nicotin.....	3.98	2.38	4.59	3.30
Malic acid.....	12.11	11.11	11.57	6.04
Citric acid.....	2.05	2.53	3.40	3.30
Oxalic acid.....	1.53	2.97	2.03	3.38
Acetic acid.....	0.42	0.29	0.43	0.22
Tannic acid.....	1.13	0.98	1.48	0.51
Nitric acid.....	1.32	0.60	1.88	0.23
Pectic acid.....	11.36	11.88	8.22	10.13
Cellulose	15.76	10.59	12.48	11.82
Ammonia.....	0.49	0.06	0.19	0.23
Soluble nitrogenous matter.	7.74	8.84	13.90	10.39
Insoluble " "	9.75	7.97	8.10	9.53
Residue and chlorophyll...	5.15	8.63	1.99	6.45
Oil	1.03	1.26	2.28	0.81
Ash	17.50	17.03	14.36	18.46
Undetermined	8.68	12.88	13.10	15.20

Among the undetermined matters are included those of a gummy or resinous composition not extracted by ether, the exact nature of which is not well understood, and the starches, sugars, pentosans and galactan.

Tobacco grown in more northern latitudes has less nicotin than the samples given in the foregoing table.

The following table shows the composition of tobacco grown in Connecticut:⁵⁹

	Upper leaves.		Short seconds.		First wrappers.	
	Unfermented, per cent.	Fermented, per cent.	Unfermented, per cent.	Fermented, per cent.	Unfermented, per cent.	Fermented, per cent.
Water.....	23.50	23.40	27.40	21.10	27.50	24.90
Pure ash....	14.89	15.27	22.85	25.25	15.84	16.22
Nicotin	2.50	1.79	0.77	0.50	1.26	1.44
Nitric acid..	1.89	1.97	2.39	2.82	2.59	2.35
Ammonia ...	0.67	0.71	0.16	0.16	0.33	0.47
Proteins.....	12.19	13.31	6.69	6.81	11.31	11.62
Fiber	7.90	8.78	7.89	8.95	9.92	10.42
Starch	3.20	3.36	2.62	3.01	2.89	3.08
Oil and fat ..	3.87	3.42	2.65	3.04	2.84	2.92
Undeterm'd .	29.39	27.99	26.28	28.36	25.52	26.88

⁵⁹ Connecticut Agricultural Experiment Station (New Haven), Annual Report, 1892 : 29.

640. Estimation of Water.—In the estimation of water in vegetable substances, as has already been noted, it is usual to dry them in the air or partial vacuum, or in an inert gas, at a temperature of 100° until a constant weight is reached. By this process, not only the water, but all substances volatile at the temperature and in the conditions mentioned are expelled. The quantity of these volatile substances in vegetable matter, as a rule, is insignificant and hence the total loss may be estimated as water. In the case of tobacco a far different condition is presented, inasmuch as the nicotin, which sometimes amounts to five per cent. of the weight of the sample, is also volatile under the conditions mentioned. It is advisable, therefore, to dry the sample of tobacco at a temperature not above 50 degrees and in a vacuum as complete as possible. Tobacco is also extremely rich in its content of crystallized mineral salts, containing often water of crystallization, and there is danger of this crystal water being lost when the sample is dried at 100° . The desiccation is conveniently made in the apparatus described on page 21. If a high vacuum be employed, *viz.*, about 25 inches of mercury, it is better not to allow the temperature to go above 40° or 45° . A rather rapid current of dry air should be allowed to pass through the apparatus for the more speedy removal of the moisture and a dish containing sulfuric acid may also be placed inside of the drying apparatus. It is possible by proceeding in this way to secure constant weight in the sample after a few hours.

641. Estimation of Nitric Acid.—The nitric acid in a sample of tobacco is most easily estimated by the ferrous chlorid process.⁶⁰

The sample is best prepared by making an alcoholic extract which is accomplished by exhausting about 25 grams of the fine tobacco powder with 200 cubic centimeters of 40 per cent. alcohol made slightly alkaline by soda lye. The mixture is boiled in a flask with a reflux condenser for about an hour. After cooling, the volume is completed to a definite quantity, and, after filtering, an aliquot part is used for the analytical process. The ammonia

⁶⁰ This work, 2 : 39 et seq.

and nicotin may be removed by distillation from an alkaline medium and the nitric acid determined in the residue.

642. Sulfuric and Hydrochloric Acids.—These two acids are determined in the ash of the sample by the usual methods. The sulfuric acid thus found represents the original sulfuric acid in combination with the bases in the mineral parts of the plant, together with that produced by the oxidation of the organic sulfur during combustion. In order to avoid all loss of sulfur during the combustion, the precautions already given should be observed. The separation of the sulfur pre-existing as sulfates from that converted into sulfates during the combustion is accomplished as previously directed.⁶¹ For ordinary purposes, this separation is not necessary.

To avoid loss of chlorin from volatilization during incineration the temperature should be kept at the lowest possible point until the mass is charred, the soluble salts extracted from the charred mass and the incineration completed as usual.

643. Oxalic, Citric and Malic Acids.—The separation or estimation of organic acids from vegetable tissues is a matter of great difficulty, especially when they exist as is usually the case, in very minute proportions. During incineration, the salts of the organic acids are converted into carbonates and the subsequent examination of the ash gives no indication of the character of the original acids. In the case of tobacco, the organic acids of chief importance, from an analytical point of view, are oxalic, citric and malic. These acids may be extracted and separated by the following process.⁶²

A mixture of 10 grams of tobacco powder and 10 grams of powdered pumice stone is impregnated with two grams of sulfuric acid solution (monohydrate) in a porcelain dish. The moderately damp, but not packed powder, is placed in a filter paper thimble and extracted for 24 hours with ether in an apparatus having only glass connections. Small amounts of organic acids may be still extracted after 24 hours but their amount is neg-

⁶¹ This work, 1, 2d Edition : 470.

⁶² Chemiker Zeitung, 1904, 28 : 775.

ligible. When the extraction is completed some water is added to the flask and the ether is distilled. The remaining aqueous solution of the organic acids is filtered and diluted to 100 cubic centimeters of which 50 cubic centimeters will serve for the determination of malic and citric acids and a like amount for oxalic acid.

The first 50 cubic centimeters is exactly neutralized with standard barium hydrate solution and with constant shaking enough 95 per cent. alcohol is added to make the alcohol content of the mixture equal 20 per cent. by volume. The precipitate of barium citrate is quickly filtered and a measured aliquot portion (as much as possible) of the filtrate, is treated with alcohol until its content is 70 per cent. by volume. The barium citrate precipitate, after washing with 20 per cent. alcohol, is almost pure and contains very little malate, while the second precipitate, after washing with 70 per cent. alcohol, is almost pure barium malate containing very little citrate. It is well to allow the barium malate to stand over night before filtering as the precipitation is then more complete and the filtration much easier. The precipitates are dried, carefully, ignited, and any barium oxid formed is converted into carbonate by the addition of a little ammonium carbonate. One gram of barium carbonate corresponds to 0.65 gram anhydrous citric acid and 0.68 gram anhydrous malic acid.

For the determination of oxalic acid the second 50 cubic centimeters is neutralized with ammonia and made up to 100 cubic centimeters. One drop of acetic acid is added and the oxalic acid precipitated with calcium acetate solution. The calcium oxalate by strong ignition is converted into calcium oxid and is weighed as such. One gram of calcium oxid equals 1.607 grams of anhydrous oxalic acid.

644. Polariscopic Determination of Malic Acid.—Goder⁶³ and Dunbar and Bacon⁶⁴ have devised a method of determining malic acid by measuring its optical activity.

⁶³ *Journal of Industrial and Engineering Chemistry*, 1911, **3** : 563-574.

⁶⁴ *Bureau of Chemistry, U. S. Department of Agriculture, Circular 76*, 1911.

The Method in Brief.—The method in brief consists in treating a portion of the neutralized⁶⁵ solution containing malic acid with uranyl acetate and polarizing it. The algebraic difference between the reading so obtained and that of the untreated solution is multiplied by the factor 0.036 to obtain the percentage of malic acid present. With the exception of *d*-tartaric acid, none of the common optically active substances interfere with the determination, and consequently they need not be removed. Uranyl acetate slightly decreases the rotation of sugars. This may give rise to an error in the estimation of small amounts of malic acid in the presence of large amounts of invert sugar. Hence, when the rotation of the original solution is negative and the approximate amount of sugar or malic acid is unknown, or when more than 10 per cent. of reducing sugars and less than 0.25 per cent. of malic acid are present, it is necessary to precipitate the malic acid and treat the filtrate with uranyl acetate also. In this case the polarization of this filtrate, instead of that of the untreated solution, is subtracted from the polarization of the solution containing both malic acid and uranyl acetate, and the difference is multiplied by 0.036.

Details of the Method.—Dilute a measured volume of the solution, usually 10 cubic centimeters, with quite a large volume of water, add phenolphthalein, and titrate with standard alkali to a decided pink color. Transfer another measured portion of the solution (75 cubic centimeters is a convenient volume) to a 100 cubic centimeter graduated flask, and add enough standard alkali, calculated from the above titration, to neutralize the acidity. A slight excess of alkali is not objectionable. If the solution is dark colored, add five or 10 cubic centimeters of alumina cream. Dilute to the mark, mix thoroughly, and filter if necessary through a folded filter.

(1) Treat about 25 cubic centimeters of the filtrate with powdered uranyl acetate, adding enough of the salt so that a small amount remains undissolved after two hours. Two and one-half grams of uranyl acetate will usually be sufficient, except in the

⁶⁵ If no mineral acids are present it is not necessary to neutralize the malic acid.

presence of large amounts of malic acid. In case all the uranium salt dissolves more should be added. Allow the mixture to stand for two hours, shaking frequently. Filter through a folded filter until clear and polarize if possible in a 200 millimeter tube. If the solution is too dark to read in a 200 millimeter tube, a 100 or 50 millimeter tube may be used. It is desirable, however, to use the longest tube possible in order to obtain the maximum rotation. This solution and reading will hereafter be designated as (1).

(2) Treat the remainder of the original filtrate with powdered normal lead acetate until no further precipitation results. Cool in an ice-bath and filter through a folded filter until clear. Warm the filtrate to room temperature and add a small crystal of lead acetate to determine whether the precipitation is complete. If no further precipitate results, remove the excess of lead completely with anhydrous sodium sulfate, filter until clear, and polarize. Designate this filtrate as solution (2) and its polarization as reading (2). Care should be taken to add no more lead acetate to the solution than is necessary for complete precipitation, as lead malate is soluble in an excess of lead acetate. Solutions which are sufficiently clear and contain less than 10 per cent. of sugar may be polarized directly without treatment with lead acetate.

(3) If reading (2) is negative treat a portion of solution (2) with uranyl acetate in the manner described under (1) and polarize. Designate this as (3). If reading (2) is positive, reading (3) need not be made.

Polarize at room temperature with white light, taking care that all solutions are polarized at the same temperature. Make at least six readings in each case and take an average of these. Calculate all readings to the basis of a 200 millimeter tube. If reading (3) is numerically less than reading (2), the latter should be discarded; otherwise use reading (2) in the subsequent calculation. Multiply the algebraic difference between this reading and reading (1) by 0.036. The product will equal the percentage

of malic acid $\left\{ \begin{array}{c} \text{CH}_2\text{COOH} \\ | \\ \text{CHOHCOOH} \end{array} \right\}$ in the solution as polarized.

645. Modification of Pratt.—The method for determining malic acid devised by Dunbar and Bacon presents some difficulties in manipulation. These, briefly, are the slowness with which the various liquids filter and the great difficulty in always obtaining solutions sufficiently clear and free from small particles for polarizing. The lead salts have a tendency to pass through the filter, especially with certain types of fruit of low acidity.

The chief cause of this slow filtration is the presence of pectin bodies, always present in fruit juices to a greater or less extent. They rapidly clog the pores of the paper, and if present in sufficient amount will entirely stop the flow. Dilution helps the filtration, but introduces errors, especially when the malic acid content is originally low. The difficulty is avoided by Pratt⁶⁶ in the following manner.

A weighed amount of juice, generally 100 grams, is placed in a 500 cubic centimeter beaker. With vigorous stirring about two or three times the volume of 95 per cent. alcohol is added. This throws out the pectin bodies, usually in such a form that after standing a few minutes they may be gathered into a coherent mass. The liquid is decanted through a filter and the precipitate washed twice with 95 per cent. alcohol. The combined filtrates are then evaporated in a current of air on the water-bath to about 75 cubic centimeters. After cooling, the solution is made up to 100 cubic centimeters in a measuring flask, using from 10 to 15 cubic centimeters of 95 per cent. alcohol and distilled water. The temperature when the volume is finally made up to the mark should be close to that at which the polariscope readings are to be taken. This solution is then treated exactly as in the original method, except that no clarification is necessary. No difficulty will be experienced in filtering, as the liquid passes rapidly through the paper and has no tendency to clog.

The addition of alcohol in making up to volume has a twofold purpose. Lead malate is less soluble in an alcoholic solution, and the necessity of cooling with ice to remove it completely is, therefore, avoided. The chief advantage, however, lies in the fact that

⁶⁶ Bureau of Chemistry, U. S. Department of Agriculture, Circular 87, 1917.

the lead salts of fruit acids are thrown out in the alcohol-containing liquid as gelatinous precipitates which filter quickly and never cause trouble by passing through the paper unless a large excess of lead acetate has been used in the precipitation. This should always be avoided by adding it in small amounts with vigorous shaking after each addition until a few crystals remain unchanged at the bottom of the flask. When the powdered anhydrous sodium sulfate is added to remove the excess of lead the same advantage is noted. In this case without the alcohol even more trouble is encountered in obtaining a filtrate entirely clear and free from small particles that render it very difficult to polarize. With the alcohol, one or at most two filtrations give a water clear solution that may be read in a 200 millimeter tube.

With that part of the solution containing the uranyl acetate a liquid is obtained which filters rapidly and can always be read in a 100 millimeter tube. In cases where the alcohol has caused some fruit color to pass into solution the addition of a tiny drop of bromin and vigorous shaking and filtering, if necessary, before reading will give satisfactory results.

The modification has been thoroughly tested in the routine analysis of a large number of fruit samples. By this means, for instance, the thick mass resulting from crushed bananas may be handled. Here the malic acid content is rather low, and the solution would have to be so diluted in order to handle it in the ordinary way that the error would be relatively large.

Another advantage of this method of procedure is that concentration of the original juice is possible. Two hundred grams may be used, and the final volume after evaporation made up as usual to 100 cubic centimeters. With juices containing but little malic acid this is a great advantage, which could not be utilized in the original method. The time required to precipitate the pectins, filter, and evaporate off the alcohol is more than compensated for by the time saved in filtering the solution, and the use of longer tubes in the polariscope increases the accuracy of the method.

646. Acetic Acid.—For the determination of the volatile acids

of the fatty series existing in tobacco, the following process, also due to Schlösing, may be followed:⁶⁷

The apparatus employed is shown in Fig. 114. Ten grams of the pulverized tobacco, moistened with water and mixed with a little powdered tartaric acid, are placed in the tube A. The two ends of the tube, A, are stoppered with asbestos or glass wool. Steam, generated in the flask, D, is passed into B. After 15 minutes, or as soon as it is certain that the contents of A have reached a temperature of 100°, the dish F, containing mercury, is placed in the position shown in the figure. The steam, by this arrangement, is forced into the lower end of A, passes into the condenser E, and the condensed water collected in C. The

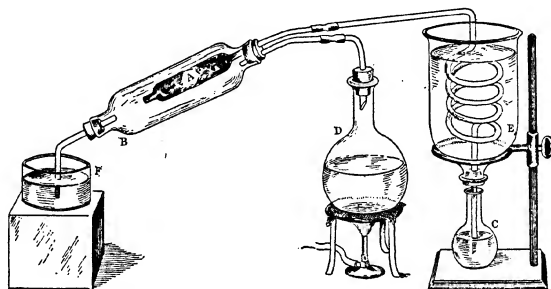


Fig. 114.—Apparatus for Acetic Acid.

operation should be so conducted as to avoid any condensation of water in B. It is advisable during the progress of the distillation, which should continue for at least twenty minutes, to neutralize from time to time the acetic acid collected in C by a set solution of dilute alkali, or, an excess of the alkaline solution may be placed in C and the part not neutralized by the acetic acid determined at the end of the distillation by titration.

647. Pectic Acid.—Under this term are included not only the pectic acid but all the other bodies of a pectose nature contained on tobacco. These bodies are of considerable interest, although they do not belong to the most important constituents. In fresh tobacco leaves are found three pectin bodies. One pectin is

⁶⁷ Kissling, *Tabakkunde*, 1893 : 64.

soluble in water, another is an insoluble pectose and the third is the pectose body forming salts with the alkalies, *i. e.*, true pectic acid. In fermented tobacco pectic acid is found chiefly in combination with lime in the ribs of the leaves, serving to give them the necessary stiffness. For the estimation of the pectin bodies (mucilage) the powdered tobacco is thoroughly extracted with cold water. An aliquot part of the aqueous extract is mixed with two volumes of strong alcohol and allowed to stand in a well closed vessel in a cool place for 24 hours. The precipitate is collected on a filter, washed with 66 per cent. alcohol, dried and weighed. The dried residue is incinerated and the amount of ash determined. In general, vegetable mucilages contain about five per cent. of ash. If more than this be found, it is due to the solution of the salts of the organic acids contained in the sample. A dried vegetable mucilage obtained as above, dissolves in water to a mucilaginous liquid which does not reduce alkaline copper solution until it has been hydrolyzed by boiling with a dilute mineral acid.⁶³

648. Tannic Acid.—This acid is separated and estimated by the processes given in paragraphs 627-634.

649. Starch and Sugar.—The unfermented leaves of tobacco contain considerable quantities of carbohydrates in addition to woody fiber, pentosans, galactan and cellulose. Among these, starch is the most important. Sugar exists in small quantities in the fresh leaf, usually not over one per cent. During fermentation according to some authorities, the starch is partially converted into sugar and the latter substance disappears under the action of the alcoholic ferments. It has been found at the Connecticut Station, however, that the starch content of the leaf does not decrease during fermentation. The starch and sugar may be determined in the fresh leaves by the methods already given.

In the manufacture of certain grades of tobacco it is customary to add a quantity of sugar. The analyst may thus be called upon to determine in some cases whether the sugar found in a

⁶³ Dragendorff, *Plant Analysis*, 1909, : 65-66. Translated by H. G. Greenish.

sample is natural or added. The occurrence of natural sugars in tobacco has been investigated at the instance of the British Treasury.⁶⁹

The natural sugars which may be found in sun dried tobaccos usually disappear entirely during the process of fermentation. It was found by the Somerset House chemists that the content of sugar in commercial tobaccos varies from none at all to over 15 per cent. A remarkable example of this variation is reported in two samples from this country, one of which, grown in Kentucky, contained no sugar, and the other grown in Virginia, 15.2 per cent.

650. Ammonia.—As has already been intimated, ammonia exists only in minute quantities in fresh tobacco leaves, but in considerable quantities after fermentation. In the estimation of ammonia, 20 grams of the tobacco powder are digested with 250 cubic centimeters of water, acidulated with sulfuric and after an hour enough water added to make the total quantity 400 cubic centimeters. After filtration, an aliquot part of the filtrate, about 200 cubic centimeters, is treated with magnesium oxid in excess and the ammonia and nicotin removed by distillation in a current of steam. The distillate is collected in dilute sulfuric acid of known strength. The total amount of the two bases is determined by titration and the quantity of base representing the nicotin, which has been determined in a separate sample, subtracted in order to obtain the weight of the ammonia.⁷⁰

The ammonia in tobacco is determined by Nessler in the following manner.⁷¹

The powdered tobacco is mixed with water and magnesium oxid and after standing for several hours it is distilled in a current of steam, the distillate received in dilute sulfuric acid and the process continued until a drop of the distillate gives no reaction for ammonia with the Nessler reagent. The excess of

⁶⁹ Sugar, 1896, 8 No. 5 : 11.

⁷⁰ Kissling, Tabakkunde, 1893 : 65.

⁷¹ Nessler, Der Tabak, 1883 : 144.

sulfuric acid in the distillate is neutralized with pure sodium carbonate and the nicotin precipitated by a neutral solution of mercuric iodid and potassium iodid. The precipitate is separated by filtration, the filtrate treated with sodium sulfid, and the ammonia again obtained by distillation with an alkali, collected in dilute solution of set sulfuric acid and determined by titration. The difference of the two determinations represents the ammonia.

651. Nicotin.—McElroy has made a study of some of the best approved methods for determining nicotin, and finds the most simple and reliable to be that proposed by Kissling.⁷² The finely powdered tobacco should be dried at a temperature not exceeding 60°, or it may be partially dried at that temperature before grinding and the final drying completed afterwards. Twenty grams of the powdered sample are intimately mixed by means of a pestle with 10 cubic centimeters of dilute alcoholic solution of soda lye, made by dissolving six grams of sodium hydroxid in 40 cubic centimeters of water and completing the volume to 100 cubic centimeters with 95 per cent. alcohol. The mass is transferred to an extraction paper cylinder, placed in an extraction apparatus and extracted for three hours with ether. The ether is nearly all removed by careful distillation, the residue mixed with 50 cubic centimeters of a very dilute soda lye solution (4 to 100) and subjected to distillation in a current of steam. The flask containing the nicotin extract should be connected with the condensing apparatus by a safety bulb as is usual in the distillation of substances containing fixed alkali. The distillation should be conducted rapidly and in such a manner that when 200 cubic centimeters of the distillate have been collected not more than 15 cubic centimeters of the liquid remain in the distillation flask. In the distillate, the nicotin is determined by titration with a set solution of dilute sulfuric acid, using rosolic acid or phenolphthalein as indicator. It is advisable to titrate each 50 cubic centimeters of the distillate as it is received and the dis-

⁷² Kissling, *Tabakkunde*, 1893 : 65-66; *Zeitschrift für analytische Chemie*, 1882. **21** : 75 ; 1883, **22** : 199-214; 1893, **32** : 277-296; 1895, **34** : 413 and 731.

tillation is continued until the last 50 cubic centimeters give no appreciable quantity of the alkaloid. In the calculations one molecule of sulfuric acid is equivalent to two molecules of nicotin according to the equation $\text{H}_2\text{SO}_4 = (\text{C}_{10}\text{H}_{14}\text{N}_2)_2$.

Polarization Method.—Popovici has based a method of detecting the quantity of nicotin in tobacco on its property of rotating the plane of polarized light.⁷³ The gyrodyne of pure nicotin is expressed by the formula $[\alpha]_D = -161^\circ.6$. When 10 parts of nicotin are mixed with 90 parts of water, this value becomes $-74^\circ.1$. By reason of this great depression in gyrodynamic value Popovici determined the relation which exists between the dilute solutions of nicotin and the number of minutes of angular rotation produced on polarization in a 200 millimeter tube. In a solution in which two grams of nicotin are contained in 50 cubic centimeters, each minute of angular rotation is found to correspond to 6.5 milligrams of nicotin. For one gram in solution in the same volume one minute of angular rotation corresponds to 5.9 milligrams and for a half gram in solution to 5.7 milligrams.

The nicotin is prepared for polarization by extracting with ether, as indicated in the previous paragraph, and the ethereal solution from 20 grams of tobacco is shaken with a concentrated solution of sodium phosphotungstate in nitric acid by means of which nicotin and ammonia are precipitated and rapidly settle. The supernatant liquid is carefully poured off and the residue made up to a volume of 50 cubic centimeters with distilled water and the nicotin freed from any of its compounds by the addition of eight grams of finely powdered barium hydroxid. In order to promote the decomposition of the nicotin compounds the mixture should be shaken at intervals for several hours. The at first blue precipitate changes into blue green and finally into yellow. It is separated by filtration and the somewhat yellow colored filtrate placed in an observation tube, polarized, the polarization calculated to minutes of angular rotation

⁷³ Zeitschrift für physiologische chemie, 1889, 13 : 445 ; 1890, 14 : 182.

and the number of minutes thus found multiplied by the nearest factor given above.

The analyst will find a description of other methods of estimating nicotine in tobacco in the periodical literature of analytical chemistry.⁷⁴

652. Toth's Method.—Toth⁷⁵ has proposed a method for nicotine determination which is especially quick and easily handled and for technical purposes sufficiently accurate.⁷⁵

The tobacco is first dried over quicklime and then ground, powdered, or crushed. Six grams of the dried sample (or 10 grams of the aqueous extract) are mixed in a porcelain dish with 10 cubic centimeters of a solution of sodium hydroxid (20 grams in 100 cubic centimeters) and sufficient plaster of Paris is then added to obtain a dry mass. The mass is transferred to a glass tube 25 centimeters long and five centimeters in diameter, 100 cubic centimeters of a mixture of equal volumes of ether and light petroleum are added, and after inserting a glass stopper the whole is well shaken. After remaining for an hour with occasional shaking, 25 cubic centimeters of the ethereal liquid are pipetted off and introduced into a shaker, and mixed with 40 to 50 cubic centimeters of water, and a drop of iodococin in excess of N/10 sulfuric acid is added and the liquid titrated with N/10 sodium hydroxid. One cubic centimeter of N/10 sulfuric acid neutralized equals 0.0162 gram of nicotine.

653. Estimation of Amid Nitrogen.—For the estimation of amid nitrogen 10 grams of the powdered tobacco are digested with 100 cubic centimeters of 40 per cent. alcohol, the extract separated by filtration, acidified with sulfuric and the albumin, peptone, nicotine and ammonia precipitated with as little phosphotungstic acid as possible. The precipitate is separated by filtration and 75 cubic centimeters of the filtrate evaporated in a thin glass or tin-foil capsule after the addition of a little barium chlorid and the nitrogen determined in the residue. The nitrogen thus obtained

⁷⁴ Journal of the American Chemical Society, 1904, **26** : 1113.

⁷⁵ Chemiker Zeitung, 1910, **34** : 10.

Revue Internationale des Falsifications 1901, **14** : 12-14.

Journal of the Chemical Society, Abstracts...., 1901, **80** : pt. 2, 363.

is that which was present in an amid state. The nitrogen present as amids, ammonia and nicotin subtracted from the total nitrogen leaves that present as protein.

654. Fractional Extraction of Tobacco.—To determine the character of the soluble constituents of tobacco it is advisable to subject it to a fractional extraction with different reagents. The reagents usually employed in the order mentioned are petroleum ether, ether, absolute alcohol, water, dilute soda lye and dilute hydrochloric acid. The extract obtained by petroleum ether contains vegetable wax, chlorophyll and its alteration products, fat, ethereal oils, and resin bodies. The extract with ether may be divided into water soluble and alcohol soluble bodies. Among the first are small quantities of glucosids and nicotin while in the alcoholic solution resin predominates.

The alcoholic extract is also divided into water soluble and alcohol soluble parts. The first contains the nicotin, which, in combination with acids, is insoluble in ether, together with tannic acid and allied bodies and also the sugar. The part insoluble in water consists chiefly of resin.

The aqueous solution contains the vegetable mucilages (pectin) soluble carbohydrates, soluble proteins and organic acids.

The dilute soda lye solution contains chiefly proteins.

The dilute hydrochloric acid solution contains the starch and the oxalic acid originally combined with lime. The extractions with dilute soda lye and dilute hydrochloric acid should be made at a boiling temperature. The residual matter consists of a mixture of carbohydrate bodies to which the term crude fiber is usually applied.

Very little is yet known concerning the waxes, resins and essential oils of tobacco and as these are doubtless of great significance, both for the aroma and for the burning qualities of tobacco, they offer a promising field for investigation.

655. Burning Qualities.—When tobacco is to be used for the manufacture of cigars, or cigarettes, or for smoking in pipes, its ability to keep burning is a matter of great importance. The tobacco, when once ignited, should burn for some time and form

a fluffy ash, free of fused mineral particles. A tobacco with good burning properties is one containing nitrates in considerable quality, not too much sugar and starch, a porous cellular structure and comparatively free of chlorin. In determining comparative burning properties the tests may be applied to the single leaf or the tobacco may be first rolled into a cigar form and burned in an artificial smoker.

In applying the test to the leaf it is important that the ignition be made with a fuse without flame, which maintains a uniform burning power. Any good slow burning fuse may be used and it is applied to the leaf in such a way that a hole may be burned in it, leaving its edges uniformly ignited. The number of seconds elapsing before the last spark is extinguished is noted. At the Connecticut Experiment Station a lighter, proposed by Nessler, is employed. It is prepared by digesting 80 grams of gum arabic in 120 cubic centimeters, and 40 grams of gum tragacanth in a quarter of a liter of water for two days, mixing the mucilaginous masses and adding 10 grams of potassium nitrate and about 350 grams of pulverized charcoal. The mixture is rolled, on a plate sprinkled with charcoal, into sticks a few inches in length and of the diameter of a cigar and dried at a gentle heat. These fuses burn slowly and without smoke and are well suited for lighting tobacco leaves. Several tests, at least six, should be made with each leaf. Leaves having a uniform burning power should be used as comparators and the number of seconds they burn be designated by 100. It is important that all the samples to be tested be exposed for a day or two to the same atmosphere in order that they may have, as nearly as possible, the same content of moisture. The burning tests, when possible, should be made both before and after fermentation. As a rule fermentation improves the burning quality of second rate leaves, but has little effect on leaves of the first quality.

656. Artificial Smoker.—For the purpose of comparing the burning properties of cigars or of leaves rolled into cigar form, the artificial smoking apparatus devised by Penfield is employed.⁷⁶

⁷⁶ Connecticut Agricultural Experiment Station (New Haven) Annual Report, 1892 : 19.

The construction of the apparatus is shown in the accompanying figure.

The lighted cigar is set in the tube at the left, so that air entering the test-tube must pass through the cigar. The test-

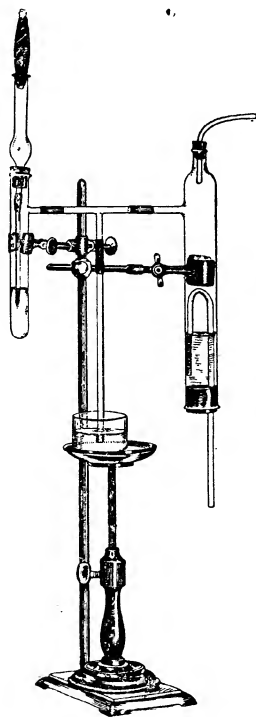


Fig. 115. Apparatus for Smoking.

tube contains enough water to seal the end of the tube carrying the cigar, and is connected with the aspirator on the right by the T tube, as shown. An arm of the T dips just beneath the surface of the liquid in the cup in the center. Water flows in a slow stream into the aspirator through the tube at the extreme right, forcing the air out through the arm of the T until the siphon begins to act. While the water is voided through the long arm

of the siphon, air enters through the cigar, the liquid rising in the T. The action of the apparatus is automatic and intermittent. When the cigar is about one-third burned, it is removed without disturbing the ash cone, and the latter examined and compared with other samples as a standard. The sealing liquid of the long arm of the T may be mercury or water. In case mercury be used, care must be taken not to immerse the open end of the T more than one millimeter therein. A "burning number" may also be obtained as suggested by Tóth.⁷⁷

FERMENTED BEVERAGES.

657. Description.—Among fermented beverages are included those drinks, containing alcohol, prepared by fermenting the sugars or starches of fruits, cereals or other agricultural products. Wine and beer, in their various forms, and cider are the chief members of this class of bodies. Koumiss, although a fermented beverage, is not included in this classification, having been noticed under dairy products. The large number of artificial drinks, made by mixing alcohol with fruit and synthesized essences, is also excluded, although the methods of analysis which are used may be applied also to them.

Fermented beverages containing less than two per cent. of alcohol are usually regarded without good reason as non-intoxicating drinks. Beers are of several varieties, and the term includes lager beer, ale, porter and stout. Distilled liquors are obtained by separating the alcohols and other volatile matters from the products of fermentation by distillation. It is not practicable here to attempt a description of the methods of preparing fermented drinks. Special works on this branch of the subject are easy of access.⁷⁸

658. Important Constituents.—Alcohol is the most important constituent of fermented beverages. The solid matters, com-

⁷⁷ *Zeitschrift für angewandte Chemie*, 1904, **17** : 1818.

⁷⁸ Buell, *The Cidermakers' Manual*, 1874 : Southby ; *Systematic Handbook of Practical Brewing*, 3d Ed., 1895 : Moritz and Morris ; *Text-Book of the Science of Brewing*, 1891.

monly called extract, which are obtained on evaporation are composed of dextrins, sugars, organic acids, nitrogenous bodies and mineral matters. Of these the dextrins and sugars form the chief part and the protein bodies nearly 10 per cent. in the case of beers made of malt and hops. In beers the bitter principles derived from hops, while not important by reason of quantity, are of the utmost consequence from a gustatory and hygienic point of view. The ash of fermented beverages varies with their nature, or with the character of the water used in making the mash. In the manufacture of beer, water containing a considerable proportion of gypsum is often used, and this substance is sometimes added in the course of manufacture, especially of wine. The presence of common salt in the ash in any notable quantity is evidence of the addition of this condiment, either to improve the taste of the beverage or to increase the thirst of the drinker. In cider the organic acids, especially malic, are of importance.

Glycerol is a product of fermentation and of the hydrolysis of the fats and oils in the substances fermented.

659. Specific Gravity.—In order to secure uniformity of expression, the specific gravity of fermented beverages is determined at about 15°.6, although that is a temperature much below the average found in American laboratories. The disadvantages of this temperature are obvious and tables are now preparing by the Bureau of Standards and the Official Chemists which will permit of the determination of alcohol at 20°. Tables for the calculation of extract from specific gravity determined at 20° will follow.

The specific gravity may be determined by a pycnometer, a small accurately graduated hydrometer, or a Westphal plummet on the analytical balance, in harmony with the directions given in paragraphs 66-67. If a pycnometer be used it should be warmed quickly to room temperature after filling and before weighing, to prevent the error due to the collection of moisture on the outside. A small hole in the cap will permit the necessary expansion in the volume of the liquid. By reason of the extrac-

tive matters held in solution, fermented beverages are usually heavier than water, even if the content of alcohol be 20 per cent. or more. On the other hand distilled liquors are lighter than water.

660. Determination of Alcohol.—The determination of the percentage of alcohol present in a solution is based on two general principles. On the one hand, and this is the base of the methods in common use, the alcohol is secured mixed only with water and its amount determined by ascertaining the specific gravity of the mixture. On the other hand the quantity of alcohol in a mixture may be determined by ascertaining the temperature of the vapors produced on boiling. This is the principle involved in the use of the ebullioscope. The latter method is not employed to any extent in this country.

Use of the Alcoholometer.—The alcoholometer usually employed is known by the name of Gay-Lussac, who first made practical use of it in the determination of alcohol. It is constructed in such a way as to read directly the volume of absolute alcohol contained in 100 volumes of the liquid at a temperature of 15°.6. The instruments employed should be carefully calibrated and thoroughly cleaned by washing with absolute alcohol before use. The stem of the instrument must be kept free from any greasy substance, and this is secured by washing it with ether. After this last washing the analyst should be careful not to touch the stem of the instrument with his fingers. It is most convenient to make the determination exactly at 15°.6, but when made at other temperatures the reading of the instrument is corrected by tables which may be found in works especially devoted to the analysis of wines.⁷⁹

In this country the alcoholometer is used to some extent, but the official method is based upon the determination of the specific gravity by an instrument constructed in every respect like the alcoholometer, but giving the specific gravity of the liquor at a given temperature instead of its percentage by volume in alcohol. The reading of the instrument having been determined at

⁷⁹ Gautier, *Sophistication et Analyse des Vins*, 1891 : 49.

a fixed temperature, the corresponding percentage of alcohol by volume or by weight is taken directly from the tables given further on.

Official Method of Distillation.—The alcoholometers employed in the official methods are calibrated to agree with those used by the officers of the Bureau of Internal Revenue. They are most conveniently constructed, carrying the thermometer scale in the

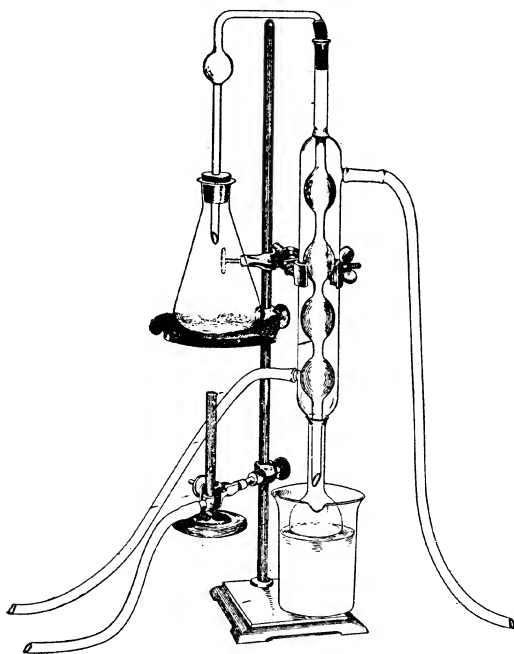


Fig. 116.—Distilling Apparatus.

same stem with that showing the specific gravity. It is highly important that the analyst assure himself of the exact calibration of the instrument before using it. Inasmuch as the volume of the distillate may not be suited in all cases to the use of a large alcoholometer, it is customary to determine the specific gravity by means of a pycnometer or the hydrostatic balance, as described further on. The distillations are conveniently conducted in a

glass apparatus shown in the accompanying figure. The manipulation is as follows:⁸⁰

Measure 100 cubic centimeters of the liquid at 15°.6 into a distilling flask of from 250 to 300 cubic centimeters capacity; add 50 cubic centimeters of water; attach the flask to a vertical condenser by means of a bent tube and distil almost 100 cubic centimeters, making up to 100 cubic centimeters volume when cooled to the standard temperature. Foaming, which sometimes occurs, especially with new wines, may be prevented by the addition of a small amount of tannin. If it be desired to determine the alcohol in wines which have undergone acetic fermentation and contain a large amount of acetic acid, 0.1 or 0.2 gram of precipitated calcium carbonate should be added. This is unnecessary, however, in wines of normal taste and odor. Determine the specific gravity of the distillate, and obtain the corresponding percentage of alcohol, by volume and grams per 100 cubic centimeters from tables. Divide the number of grams of alcohol per 100 cubic centimeters of the distillate (corresponding to the specific gravity in the appended table) by the weight (specific gravity \times 100) of sample to obtain the per cent. of alcohol by weight.

Alcohol by Weight (in Distilled Liquors).—Put in a distilling flask 20-25 grams of the sample, dilute with 100 cubic centimeters of water, distill nearly 100 cubic centimeters and either weigh distillate or make to volume at standard temperature, and determine the specific gravity. Obtain the percentage of alcohol by weight corresponding to the specific gravity from the table; multiply this figure by the weight of the distillate and divide by the weight of the sample to obtain the per cent. by weight of alcohol.

Alcohol by Volume—Official.—From the specific gravity of the distillate obtained find the percentage of alcohol by volume from the table. Multiply this figure by the volume of distillate (calculated from the specific gravity) and divide by the volume of the sample, thus obtaining the percentage of alcohol by volume in the original sample.

⁸⁰ Bureau of Chemistry, U. S. Department of Agriculture Bulletin 107 (revised), 1912 : 83.

661. Calculating Results.—The specific gravity of the alcoholic distillate having been determined by any approved method and corrected to a standard temperature, the corresponding per cents. of alcohol by volume and by weight are found by consulting the following table.⁸¹ If, for example, the corrected specific gravity be exactly that given in any figure of the table the corresponding per cents. are directly read. If the specific gravity found fall between two numbers in the table the corresponding per cents. are determined by interpolation.

⁸¹ Bureau of Chemistry, U. S. Department of Agriculture, Bulletin 107 (revised), 1912, 203 : et seq.

TABLE SHOWING PERCENTAGE OF ALCOHOL BY WEIGHT AND BY VOLUME AND GRAMS PER 100 CUBIC CENTIMETER

Percentage of Alcohol.

[Recalculated from the determinations of Gilpin, Drinkwater, and Squibb]

Specific gravity at 60° F.	Alcohol.			Specific gravity at 60° F.	Alcohol.		
	Per cent. by volume	Per cent. by weight	Grams per 100 cubic centimeters		Per cent. by volume.	Per cent. by weight.	Grams per 100 cubic centimeters.
1.00000	0.00	0.00	0.00	0.99738	1.75	1.39	1.39
0.99992	0.05	0.04	0.04	0.99731	1.80	1.43	1.43
0.99984	0.10	0.08	0.08	0.99723	1.85	1.47	1.47
0.99976	0.15	0.12	0.12	0.99716	1.90	1.51	1.51
0.99968	0.20	0.16	0.16	0.99708	1.95	1.55	1.55
0.99961	0.25	0.20	0.20	0.99701	2.00	1.59	1.59
0.99953	0.30	0.24	0.24	0.99694	2.05	1.63	1.62
0.99945	0.35	0.28	0.28	0.99687	2.10	1.67	1.66
0.99937	0.40	0.32	0.32	0.99679	2.15	1.71	1.70
0.99930	0.45	0.36	0.36	0.99672	2.20	1.75	1.74
0.99923	0.50	0.40	0.40	0.99665	2.25	1.79	1.78
0.99915	0.55	0.44	0.44	0.99658	2.30	1.83	1.82
0.99907	0.60	0.48	0.48	0.99651	2.35	1.87	1.86
0.99900	0.65	0.52	0.52	0.99643	2.40	1.91	1.90
0.99892	0.70	0.56	0.56	0.99636	2.45	1.95	1.94
0.99884	0.75	0.60	0.60	0.99629	2.50	1.99	1.98
0.99877	0.80	0.64	0.64	0.99622	2.55	2.03	2.02
0.99869	0.85	0.67	0.67	0.99615	2.60	2.07	2.06
0.99861	0.90	0.71	0.71	0.99607	2.65	2.11	2.10
0.99854	0.95	0.75	0.75	0.99600	2.70	2.15	2.14
0.99849	1.00	0.79	0.79	0.99593	2.75	2.19	2.18
0.99842	1.05	0.83	0.83	0.99586	2.80	2.23	2.22
0.99834	1.10	0.87	0.87	0.99579	2.85	2.27	2.26
0.99827	1.15	0.91	0.91	0.99571	2.90	2.31	2.30
0.99819	1.20	0.95	0.95	0.99564	2.95	2.35	2.34
0.99812	1.25	0.99	0.99	0.99557	3.00	2.39	2.38
0.99805	1.30	1.03	1.03	0.99550	3.05	2.43	2.42
0.99797	1.35	1.07	1.07	0.99543	3.10	2.47	2.46
0.99790	1.40	1.11	1.11	0.99536	3.15	2.51	2.50
0.99782	1.45	1.15	1.15	0.99529	3.20	2.55	2.54
0.99775	1.50	1.19	1.19	0.99522	3.25	2.59	2.58
0.99768	1.55	1.23	1.23	0.99515	3.30	2.64	2.62
0.99760	1.60	1.27	1.27	0.99508	3.35	2.68	2.66
0.99753	1.65	1.31	1.31	0.99501	3.40	2.72	2.70
0.99745	1.70	1.35	1.35	0.99494	3.45	2.76	2.74

PERCENTAGE OF ALCOHOL, BY WEIGHT, ETC.—*Continued.*

Specific gravity at 68°F.	Alcohol.			Specific gravity at 68°F.	Alcohol.		
	Per cent. by volume.	Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by volume.	Per cent. by weight.	Grams per 100 cubic centimeters.
0.99487	3.50	2.80	2.78	0.99215	5.50	4.40	4.37
0.99480	3.55	2.84	2.82	0.99208	5.55	4.44	4.40
0.99473	3.60	2.88	2.86	0.99202	5.60	4.48	4.44
0.99466	3.65	2.92	2.90	0.99195	5.65	4.52	4.48
0.99459	3.70	2.96	2.94	0.99189	5.70	4.56	4.52
0.99452	3.75	3.00	2.98	0.99182	5.75	4.60	4.56
0.99445	3.80	3.04	3.02	0.99175	5.80	4.64	4.60
0.99438	3.85	3.08	3.06	0.99169	5.85	4.68	4.64
0.99431	3.90	3.12	3.10	0.99162	5.90	4.72	4.68
0.99424	3.95	3.16	3.14	0.99156	5.95	4.76	4.72
0.99417	4.00	3.20	3.18	0.99149	6.00	4.80	4.76
0.99410	4.05	3.24	3.22	0.99143	6.05	4.84	4.80
0.99403	4.10	3.28	3.26	0.99136	6.10	4.88	4.84
0.99397	4.15	3.32	3.30	0.99130	6.15	4.92	4.88
0.99390	4.20	3.36	3.34	0.99123	6.20	4.96	4.92
0.99383	4.25	3.40	3.38	0.99117	6.25	5.00	4.96
0.99376	4.30	3.44	3.42	0.99111	6.30	5.05	5.00
0.99369	4.35	3.48	3.46	0.99104	6.35	5.09	5.04
0.99363	4.40	3.52	3.50	0.99098	6.40	5.13	5.08
0.99356	4.45	3.56	3.54	0.99091	6.45	5.17	5.12
0.99349	4.50	3.60	3.58	0.99085	6.50	5.21	5.16
0.99342	4.55	3.64	3.62	0.99079	6.55	5.25	5.20
0.99335	4.60	3.68	3.66	0.99072	6.60	5.29	5.24
0.99329	4.65	3.72	3.70	0.99066	6.65	5.33	5.28
0.99322	4.70	3.76	3.74	0.99059	6.70	5.37	5.32
0.99315	4.75	3.80	3.77	0.99053	6.75	5.41	5.36
0.99308	4.80	3.84	3.81	0.99047	6.80	5.45	5.40
0.99301	4.85	3.88	3.85	0.99040	6.85	5.49	5.44
0.99295	4.90	3.92	3.89	0.99034	6.90	5.53	5.48
0.99288	4.95	3.96	3.93	0.99027	6.95	5.57	5.52
0.99281	5.00	4.00	3.97	0.99021	7.00	5.61	5.56
0.99274	5.05	4.04	4.01	0.99015	7.05	5.65	5.60
0.99268	5.10	4.08	4.05	0.99009	7.10	5.69	5.64
0.99261	5.15	4.12	4.09	0.99002	7.15	5.73	5.68
0.99255	5.20	4.16	4.13	0.98996	7.20	5.77	5.72
0.99248	5.25	4.20	4.17	0.98990	7.25	5.81	5.76
0.99241	5.30	4.24	4.21	0.98984	7.30	5.86	5.80
0.99235	5.35	4.28	4.25	0.98978	7.35	5.90	5.84
0.99228	5.40	4.32	4.29	0.98971	7.40	5.94	5.88
0.99222	5.45	4.36	4.33	0.98965	7.45	5.98	5.92

PERCENTAGE OF ALCOHOL, BY WEIGHT, ETC.—*Continued.*

Specific gravity at 60° F.	Alcohol.			Specific gravity at 60° F.	Alcohol.		
	Per cent. by volume.	Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by volume.	Per cent. by weight.	Grams per 100 cubic centimeters.
0.98939	7.50	6.02	5.96	0.98719	9.50	7.64	7.54
0.98953	7.55	6.06	6.00	0.98713	9.55	7.68	7.58
0.98947	7.60	6.10	6.04	0.98707	9.60	7.72	7.62
0.98940	7.65	6.14	6.07	0.98701	9.65	7.76	7.66
0.98934	7.70	6.18	6.11	0.98695	9.70	7.80	7.70
0.98928	7.75	6.22	6.15	0.98689	9.75	7.84	7.74
0.98922	7.80	6.26	6.19	0.98683	9.80	7.88	7.78
0.98916	7.85	6.30	6.23	0.98678	9.85	7.92	7.82
0.98909	7.90	6.34	6.27	0.98672	9.90	7.96	7.85
0.98903	7.95	6.38	6.31	0.98666	9.95	8.00	7.89
0.98897	8.00	6.42	6.35	0.98660	10.00	8.04	7.93
0.98891	8.05	6.46	6.39	0.98654	10.05	8.08	7.97
0.98885	8.10	6.50	6.43	0.98649	10.10	8.12	8.01
0.98879	8.15	6.54	6.47	0.98643	10.15	8.16	8.05
0.98873	8.20	6.58	6.51	0.98637	10.20	8.20	8.09
0.98867	8.25	6.62	6.55	0.98632	10.25	8.24	8.13
0.98861	8.30	6.67	6.59	0.98626	10.30	8.29	8.17
0.98855	8.35	6.71	6.63	0.98620	10.35	8.33	8.21
0.98849	8.40	6.75	6.67	0.98614	10.40	8.37	8.25
0.98843	8.45	6.79	6.71	0.98609	10.45	8.41	8.29
0.98837	8.50	6.83	6.75	0.98603	10.50	8.45	8.33
0.98831	8.55	6.87	6.79	0.98597	10.55	8.49	8.37
0.98825	8.60	6.91	6.83	0.98592	10.60	8.53	8.41
0.98819	8.65	6.95	6.87	0.98586	10.65	8.57	8.45
0.98813	8.70	6.99	6.91	0.98580	10.70	8.61	8.49
0.98807	8.75	7.03	6.95	0.98575	10.75	8.65	8.53
0.98801	8.80	7.07	6.99	0.98569	10.80	8.70	8.57
0.98795	8.85	7.11	7.03	0.98563	10.85	8.74	8.61
0.98789	8.90	7.15	7.07	0.98557	10.90	8.78	8.65
0.98783	8.95	7.19	7.11	0.98552	10.95	8.82	8.69
0.98777	9.00	7.23	7.14	0.98546	11.00	8.86	8.73
0.98771	9.05	7.27	7.18	0.98540	11.05	8.90	8.77
0.98765	9.10	7.31	7.22	0.98535	11.10	8.94	8.81
0.98759	9.15	7.35	7.26	0.98529	11.15	8.98	8.85
0.98754	9.20	7.39	7.30	0.98524	11.20	9.02	8.89
0.98748	9.25	7.43	7.34	0.98518	11.25	9.07	8.93
0.98742	9.30	7.48	7.38	0.98513	11.30	9.11	8.97
0.98736	9.35	7.52	7.42	0.98507	11.35	9.15	9.01
0.98730	9.40	7.56	7.46	0.98502	11.40	9.19	9.05
0.98724	9.45	7.60	7.50	0.98496	11.45	9.23	9.09

PERCENTAGE OF ALCOHOL, BY WEIGHT, ETC.—*Continued.*

Specific gravity at 60°F.	Alcohol.			Specific gravity at 60°F.	Alcohol.		
	Per cent. by volume.	Per cent. by weight.	Grams per 100 cubic centimeter.		Per cent. by volume.	Per cent. by weight.	Grams per 100 cubic centimeters.
0.98491	11.50	9.27	9.13	0.98273	13.50	10.90	10.71
0.98485	11.55	9.31	9.17	0.98267	13.55	10.94	10.75
0.98479	11.60	9.35	9.21	0.98262	13.60	10.98	10.79
0.98474	11.65	9.39	9.25	0.98256	13.65	11.02	10.83
0.98468	11.70	9.43	9.29	0.98251	13.70	11.06	10.87
0.98463	11.75	9.47	9.32	0.98246	13.75	11.11	10.91
0.98457	11.80	9.51	9.36	0.98240	13.80	11.15	10.95
0.98452	11.85	9.55	9.40	0.98235	13.85	11.19	10.99
0.98446	11.90	9.59	9.44	0.98230	13.90	11.23	11.03
0.98441	11.95	9.63	9.48	0.98224	13.95	11.27	11.07
0.98435	12.00	9.67	9.52	0.98219	14.00	11.31	11.11
0.98430	12.05	9.71	9.56	0.98214	14.05	11.35	11.15
0.98424	12.10	9.75	9.60	0.98209	14.10	11.39	11.19
0.98419	12.15	9.79	9.64	0.98203	14.15	11.43	11.23
0.98413	12.20	9.83	9.68	0.98198	14.20	11.47	11.27
0.98408	12.25	9.87	9.72	0.98193	14.25	11.52	11.31
0.98402	12.30	9.92	9.76	0.98188	14.30	11.56	11.35
0.98397	12.35	9.96	9.80	0.98182	14.35	11.60	11.39
0.98391	12.40	10.00	9.84	0.98177	14.40	11.64	11.43
0.98386	12.45	10.04	9.88	0.98172	14.45	11.68	11.47
0.98381	12.50	10.08	9.92	0.98167	14.50	11.72	11.51
0.98375	12.55	10.12	9.96	0.98161	14.55	11.76	11.55
0.98370	12.60	10.16	10.00	0.98156	14.60	11.80	11.59
0.98364	12.65	10.20	10.03	0.98151	14.65	11.84	11.63
0.98359	12.70	10.24	10.07	0.98146	14.70	11.88	11.67
0.98353	12.75	10.28	10.11	0.98140	14.75	11.93	11.71
0.98348	12.80	10.33	10.15	0.98135	14.80	11.97	11.75
0.98342	12.85	10.37	10.19	0.98130	14.85	12.01	11.79
0.98337	12.90	10.41	10.23	0.98125	14.90	12.05	11.82
0.98331	12.95	10.45	10.27	0.98119	14.95	12.09	11.86
0.98326	13.00	10.49	10.31	0.98114	15.00	12.13	11.90
0.98321	13.05	10.53	10.35	0.98108	15.05	12.17	11.94
0.98315	13.10	10.57	10.39	0.98104	15.10	12.21	11.98
0.98310	13.15	10.61	10.43	0.98099	15.15	12.25	12.02
0.98305	13.20	10.65	10.47	0.98093	15.20	12.29	12.06
0.98299	13.25	10.69	10.51	0.98088	15.25	12.33	12.10
0.98294	13.30	10.74	10.55	0.98083	15.30	12.38	12.14
0.98289	13.35	10.78	10.59	0.98078	15.35	12.42	12.18
0.98283	13.40	10.82	10.63	0.98073	15.40	12.46	12.22
0.98278	13.45	10.86	10.67	0.98068	15.45	12.50	12.26

PERCENTAGE OF ALCOHOL BY WEIGHT, ETC.—*Continued.*

Specific gravity at 59°F.	Alcohol.			Specific gravity at 59°F.	Alcohol.		
	Per cent. by volume.	Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by volume.	Per cent. by weight.	Grams per 100 cubic centimeters.
0.98063	15.50	12.54	12.30	0.97859	17.50	14.19	13.89
0.98057	15.55	12.58	12.34	0.97853	17.55	14.23	13.92
0.98052	15.60	12.62	12.37	0.97848	17.60	14.27	13.96
0.98047	15.65	12.66	12.41	0.97843	17.65	14.31	14.00
0.98042	15.70	12.70	12.45	0.97838	17.70	14.35	14.04
0.98037	15.75	12.75	12.49	0.97833	17.75	14.40	14.08
0.98032	15.80	12.79	12.53	0.97828	17.80	14.44	14.12
0.98026	15.85	12.83	12.57	0.97823	17.85	14.48	14.16
0.98021	15.90	12.87	12.61	0.97818	17.90	14.52	14.20
0.98016	15.95	12.91	12.65	0.97813	17.95	14.56	14.24
0.98011	16.00	12.95	12.69	0.97808	18.00	14.60	14.28
0.98005	16.05	12.99	12.73	0.97803	18.05	14.64	14.32
0.98001	16.10	13.03	12.77	0.97798	18.10	14.68	14.36
0.97996	16.15	13.08	12.81	0.97793	18.15	14.73	14.40
0.97991	16.20	13.12	12.85	0.97788	18.20	14.77	14.44
0.97986	16.25	13.16	12.89	0.97783	18.25	14.81	14.48
0.97980	16.30	13.20	12.93	0.97778	18.30	14.85	14.52
0.97975	16.35	13.24	12.97	0.97773	18.35	14.89	14.56
0.97970	16.40	13.29	13.01	0.97768	18.40	14.94	14.60
0.97965	16.45	13.33	13.05	0.97763	18.45	14.98	14.64
0.97960	16.50	13.37	13.09	0.97758	18.50	15.02	14.68
0.97955	16.55	13.41	13.13	0.97753	18.55	15.06	14.72
0.97950	16.60	13.45	13.17	0.97748	18.60	15.10	14.76
0.97945	16.65	13.49	13.21	0.97743	18.65	15.14	14.80
0.97940	16.70	13.53	13.25	0.97738	18.70	15.18	14.84
0.97935	16.75	13.57	13.29	0.97733	18.75	15.22	14.88
0.97929	16.80	13.62	13.33	0.97728	18.80	15.27	14.92
0.97924	16.85	13.66	13.37	0.97723	18.85	15.31	14.96
0.97919	16.90	13.70	13.41	0.97718	18.90	15.38	15.00
0.97914	16.95	13.74	13.45	0.97713	18.95	15.39	15.04
0.97909	17.00	13.78	13.49	0.97708	19.00	15.43	15.08
0.97904	17.05	13.82	13.53	0.97703	19.05	15.47	15.11
0.97899	17.10	13.86	13.57	0.97698	19.10	15.51	15.15
0.97894	17.15	13.90	13.61	0.97693	19.15	15.55	15.19
0.97889	17.20	13.94	13.65	0.97688	19.20	15.59	15.23
0.97884	17.25	13.98	13.69	0.97683	19.25	15.63	15.27
0.97879	17.30	14.03	13.73	0.97678	19.30	15.68	15.31
0.97874	17.35	14.07	13.77	0.97673	19.35	15.72	15.35
0.97869	17.40	14.11	13.81	0.97668	19.40	15.76	15.39
0.97864	17.45	14.15	13.85	0.97663	19.45	15.80	15.43

PERCENTAGE OF ALCOHOL BY WEIGHT, ETC.—*Continued.*

Specific gravity at 60°F.	Alcohol.			Specific gravity at 60°F.	Alcohol.		
	Per cent. by volume	Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by volume.	Per cent. by weight	Grams per 100 cubic centimeters
0.97658	19.50	15.84	15.47	0.97457	21.50	17.51	17.66
0.97653	19.55	15.88	15.51	0.97451	21.55	17.55	17.10
0.97648	19.60	15.93	15.55	0.97446	21.60	17.59	17.14
0.97643	19.65	15.97	15.59	0.97441	21.65	17.63	17.18
0.97638	19.70	16.01	15.63	0.97436	21.70	17.67	17.22
0.97633	19.75	16.05	15.67	0.97431	21.75	17.71	17.26
0.97628	19.80	16.09	15.71	0.97426	21.80	17.76	17.30
0.97623	19.85	16.14	15.75	0.97421	21.85	17.80	17.34
0.97618	19.90	16.18	15.79	0.97416	21.90	17.84	17.38
0.97613	19.95	16.22	15.83	0.97411	21.95	17.88	17.42
0.97608	20.00	16.26	15.87	0.97406	22.00	17.92	17.46
0.97603	20.05	16.30	15.91	0.97401	22.05	17.96	17.50
0.97598	20.10	16.34	15.95	0.97396	22.10	18.00	17.54
0.97593	20.15	16.38	15.99	0.97391	22.15	18.05	17.58
0.97588	20.20	16.42	16.03	0.97386	22.20	18.09	17.62
0.97583	20.25	16.46	16.06	0.97381	22.25	18.13	17.66
0.97578	20.30	16.51	16.10	0.97375	22.30	18.17	17.70
0.97573	20.35	16.58	16.14	0.97370	22.35	18.21	17.74
0.97568	20.40	16.59	16.18	0.97365	22.40	18.26	17.78
0.97563	20.45	16.63	16.22	0.97360	22.45	18.30	17.82
0.97558	20.50	16.67	16.26	0.97355	22.50	18.34	17.86
0.97552	20.55	16.71	16.30	0.97350	22.55	18.38	17.90
0.97547	20.60	16.75	16.34	0.97345	22.60	18.42	17.94
0.97542	20.65	16.80	16.38	0.97340	22.65	18.47	17.98
0.97537	20.70	16.84	16.42	0.97335	22.70	18.51	18.02
0.97532	20.75	16.88	16.46	0.97330	22.75	18.55	18.06
0.97527	20.80	16.92	16.50	0.97324	22.80	18.59	18.10
0.97522	20.85	16.96	16.54	0.97319	22.85	18.63	18.14
0.97517	20.90	17.01	16.58	0.97314	22.90	18.68	18.18
0.97512	20.95	17.05	16.62	0.97309	22.95	18.72	18.22
0.97507	21.00	17.09	16.66	0.97304	23.00	18.76	18.26
0.97502	21.05	17.13	16.70	0.97299	23.05	18.80	18.29
0.97497	21.10	17.17	16.74	0.97294	23.10	18.84	18.33
0.97492	21.15	17.22	16.78	0.97289	23.15	18.88	18.37
0.97487	21.20	17.26	16.82	0.97283	23.20	18.92	18.41
0.97482	21.25	17.30	16.86	0.97278	23.25	18.96	18.45
0.97477	21.30	17.34	16.90	0.97273	23.30	19.01	18.49
0.97472	21.35	17.38	16.94	0.97268	23.35	19.05	18.53
0.97467	21.40	17.43	16.98	0.97263	23.40	19.09	18.57
0.97462	21.45	17.47	17.02	0.97258	23.45	19.13	18.61

PERCENTAGE OF ALCOHOL BY WEIGHT, ETC.—Continued.

Specific gravity at 60°F.	Alcohol.			Specific gravity at 60°F.	Alcohol.		
	Per cent. by volume.	Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by volume.	Per cent. by weight.	Grams per 100 cubic centimeters.
0.97253	23.50	19.17	18.65	0.97044	25.50	20.85	20.24
0.97247	23.55	19.21	18.69	0.97039	25.55	20.89	20.28
0.97242	23.60	19.25	18.73	0.97033	25.60	20.93	20.32
0.97237	23.65	19.30	18.77	0.97028	25.65	20.98	20.36
0.97232	23.70	19.34	18.81	0.97023	25.70	21.02	20.40
0.97227	23.75	19.38	18.84	0.97018	25.75	21.06	20.44
0.97222	23.80	19.42	18.88	0.97012	25.80	21.10	20.47
0.97216	23.85	19.46	18.92	0.97007	25.85	21.14	20.51
0.97211	23.90	19.51	18.96	0.97001	25.90	21.19	20.55
0.97206	23.95	19.55	19.00	0.96996	25.95	21.23	20.59
0.97201	24.00	19.59	19.04	0.96991	26.00	21.27	20.63
0.97196	24.05	19.63	19.08	0.96986	26.05	21.31	20.67
0.97191	24.10	19.67	19.12	0.96980	26.10	21.35	20.71
0.97185	24.15	19.72	19.16	0.96975	26.15	21.40	20.75
0.97180	24.20	19.76	19.20	0.96969	26.20	21.44	20.79
0.97175	24.25	19.80	19.24	0.96964	26.25	21.48	20.83
0.97170	24.30	19.84	19.28	0.96959	26.30	21.52	20.87
0.97165	24.35	19.88	19.32	0.96953	26.35	21.56	20.91
0.97159	24.40	19.93	19.36	0.96949	26.40	21.61	20.95
0.97154	24.45	19.97	19.40	0.96942	26.45	21.65	20.99
0.97149	24.50	20.01	19.44	0.96939	26.50	21.69	21.03
0.97144	24.55	20.05	19.48	0.96932	26.55	21.73	21.07
0.97139	24.60	20.09	19.52	0.96926	26.60	21.77	21.11
0.97133	24.65	20.14	19.56	0.96921	26.65	21.82	21.15
0.97128	24.70	20.18	19.60	0.96915	26.70	21.86	21.19
0.97123	24.75	20.22	19.64	0.96910	26.75	21.90	21.23
0.97118	24.80	20.26	19.68	0.96905	26.80	21.94	21.27
0.97113	24.85	20.30	19.72	0.96899	26.85	21.98	21.31
0.97107	24.90	20.35	19.76	0.96894	26.90	22.03	21.35
0.97102	24.95	20.36	19.80	0.96888	26.95	22.07	21.39
0.97097	25.00	20.43	19.84	0.96883	27.00	22.11	21.43
0.97092	25.05	20.47	19.88	0.96877	27.05	22.15	21.47
0.97086	25.10	20.51	19.92	0.96872	27.10	22.20	21.51
0.97081	25.15	20.56	19.96	0.96866	27.15	22.24	21.55
0.97076	25.20	20.60	20.00	0.96861	27.20	22.28	21.59
0.97071	25.25	20.64	20.04	0.96855	27.25	22.33	21.63
0.97065	25.30	20.68	20.08	0.96850	27.30	22.37	21.67
0.97060	25.35	20.72	20.12	0.96844	27.35	22.41	21.71
0.97055	25.40	20.77	20.16	0.96839	27.40	22.45	21.75
0.97049	25.45	20.81	20.20	0.96833	27.45	25.50	21.79

PERCENTAGE OF ALCOHOL, BY WEIGHT, ETC.—*Continued.*

Specific gravity at 60°F.	Alcohol.			Specific gravity at 60°F.	Alcohol.		
	Per cent. by volume	Per cent. by weight.	Grams per 100 cubic centi. meters.		Per cent. by volume.	Per cent. by weight.	Grams per 100 cubic centi. meters.
0.96828	27.50	22.54	21.83	0.96600	29.50	24.23	23.41
0.96822	27.55	22.58	21.86	0.96594	29.55	24.27	23.45
0.96816	27.60	22.62	21.90	0.96587	29.60	24.32	23.49
0.96811	27.65	22.67	21.94	0.96582	29.65	24.36	23.53
0.96805	27.70	22.71	21.98	0.96576	29.70	24.40	23.57
0.96800	27.75	22.75	22.02	0.96570	29.75	24.45	23.61
0.96794	27.80	22.79	22.06	0.96564	29.80	24.49	23.65
0.96789	27.85	22.83	22.10	0.96559	29.85	24.53	23.69
0.96783	27.90	22.88	22.14	0.96553	29.90	24.57	23.73
0.96778	27.95	22.92	22.18	0.96547	29.95	24.62	23.77
0.96772	28.00	22.96	22.22	0.96541	30.00	24.66	23.81
0.96766	28.05	23.00	22.26	0.96535	30.05	24.70	23.85
0.96761	28.10	23.04	22.30	0.96529	30.10	24.74	23.89
0.96755	28.15	23.09	22.34	0.96523	30.15	24.79	23.93
0.96749	28.20	23.13	22.38	0.96517	30.20	24.83	23.97
0.96744	28.25	23.17	22.42	0.96511	30.25	24.87	24.01
0.96738	28.30	23.21	22.45	0.96505	30.30	24.91	24.04
0.96732	28.35	23.25	22.49	0.96499	30.35	24.95	24.08
0.96726	28.40	23.30	22.53	0.96493	30.40	25.00	24.12
0.96721	28.45	23.34	22.57	0.96487	30.45	25.04	24.16
0.96715	28.50	23.38	22.61	0.96481	30.50	25.08	24.20
0.96709	28.55	23.42	22.65	0.96475	30.55	25.12	24.24
0.96704	28.60	23.47	22.69	0.96469	30.60	25.17	24.28
0.96698	28.65	23.51	22.73	0.96463	30.65	25.21	24.32
0.96692	28.70	23.55	22.77	0.96457	30.70	25.25	24.36
0.96687	28.75	23.60	22.81	0.96451	30.75	25.30	24.40
0.96681	28.80	23.64	22.85	0.96445	30.80	25.34	24.44
0.96675	28.85	23.68	22.89	0.96439	30.85	25.38	24.48
0.96669	28.90	23.72	22.93	0.96433	30.90	25.42	24.52
0.96664	28.95	23.77	22.97	0.96427	30.95	25.47	24.56
0.96658	29.00	23.81	23.01	0.96421	31.00	25.51	24.60
0.96652	29.05	23.85	23.05	0.96415	31.05	25.55	24.64
0.96646	29.10	23.89	23.09	0.96409	31.10	25.60	24.68
0.96640	29.15	23.94	23.13	0.96403	31.15	25.64	24.72
0.96635	29.20	23.98	23.17	0.96396	31.20	25.68	24.76
0.96629	29.25	24.02	23.21	0.96390	31.25	25.73	24.80
0.96623	29.30	24.06	23.25	0.96384	31.30	25.77	24.84
0.96617	29.35	24.10	23.29	0.96378	31.35	25.81	24.88
0.96611	29.40	24.15	23.33	0.96372	31.40	25.85	24.92
0.96605	29.45	24.19	23.37	0.96366	31.45	25.90	24.96

PERCENTAGE OF ALCOHOL, BY WEIGHT, ETC.--*Continued.*

Specific gravity at 60°F.	Alcohol.			Specific gravity at 60°F.	Alcohol.		
	Per cent. by volume.	Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by volume.	Per cent. by weight.	Grams per 100 cubic centimeters.
0.96360	31.50	25.94	25.00	0.96108	33.50	27.66	26.59
0.96353	31.55	25.98	25.04	0.96101	33.55	27.70	26.63
0.96347	31.60	26.03	25.08	0.96095	33.60	27.75	26.67
0.96341	31.65	26.07	25.12	0.96088	33.65	27.79	26.71
0.96335	31.70	26.11	25.16	0.96082	33.70	27.83	26.75
0.96329	31.75	26.16	25.20	0.96075	33.75	27.88	26.79
0.96323	31.80	26.20	25.24	0.96069	33.80	27.92	26.82
0.96316	31.85	26.24	25.28	0.96062	33.85	27.96	26.86
0.96310	31.90	26.28	25.32	0.96056	33.90	28.00	26.90
0.96304	31.95	26.33	25.36	0.96049	33.95	28.05	26.94
0.96298	32.00	26.37	25.40	0.96043	34.00	28.09	26.98
0.96292	32.05	26.41	25.44	0.96036	34.05	28.13	27.02
0.96285	32.10	26.46	25.48	0.96030	34.10	28.18	27.06
0.96279	32.15	26.50	25.52	0.96023	34.15	28.22	27.10
0.96273	32.20	26.54	25.56	0.96016	34.20	28.26	27.14
0.96267	32.25	26.59	25.60	0.96010	34.25	28.31	27.18
0.96260	32.30	26.63	25.64	0.96003	34.30	28.35	27.22
0.96254	32.35	26.67	25.68	0.95996	34.35	28.39	27.26
0.96248	32.40	26.71	25.71	0.95990	34.40	28.43	27.30
0.96241	32.45	26.76	25.75	0.95983	34.45	28.48	27.34
0.96235	32.50	26.80	25.79	0.95977	34.50	28.52	27.38
0.96229	32.55	26.84	25.83	0.95970	34.55	28.56	27.42
0.96222	32.60	26.89	25.87	0.95963	34.60	28.61	27.46
0.96216	32.65	26.93	25.91	0.95957	34.65	28.65	27.50
0.96210	32.70	26.97	25.95	0.95950	34.70	28.70	27.54
0.96204	32.75	27.02	25.99	0.95943	34.75	28.74	27.58
0.96197	32.80	27.06	26.03	0.95937	34.80	28.78	27.62
0.96191	32.85	27.10	26.07	0.95930	34.85	28.83	27.66
0.96185	32.90	27.14	26.11	0.95923	34.90	28.87	27.70
0.96178	32.95	27.19	26.15	0.95917	34.95	28.92	27.74
0.96172	33.00	27.23	26.19	0.95910	35.00	28.96	27.78
0.96166	33.05	27.27	26.23	0.95903	35.05	29.00	27.82
0.96159	33.10	27.32	26.27	0.95896	35.10	29.05	27.86
0.96153	33.15	27.36	26.31	0.95889	35.15	29.09	27.90
0.96146	33.20	27.40	26.35	0.95883	35.20	29.13	27.94
0.96140	33.25	27.45	26.39	0.95876	35.25	29.18	27.98
0.96133	33.30	27.49	26.43	0.95869	35.30	29.22	28.02
0.96127	33.35	27.53	26.47	0.95862	35.35	29.26	28.05
0.96120	33.40	27.57	26.51	0.95855	35.40	29.30	28.09
0.96114	33.45	27.62	26.55	0.95848	35.45	29.35	28.13

PERCENTAGE OF ALCOHOL BY WEIGHT, ETC.—*Continued.*

Specific gravity at 60°F.	Alcohol.			Specific gravity at 60°F.	Alcohol.		
	Per cent. by volume.	Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by volume.	Per cent. by weight.	Grams per 100 cubic centimeters.
0.95842	35.50	29.40	28.17	0.95560	37.50	31.14	29.76
0.95835	35.55	29.43	28.21	0.95552	37.55	31.18	29.80
0.95828	35.60	29.48	28.25	0.95545	37.60	31.23	29.84
0.95821	35.65	29.52	28.29	0.95538	37.65	31.27	29.88
0.95814	35.70	29.57	28.33	0.95531	37.70	31.32	29.92
0.95807	35.75	29.61	28.37	0.95523	37.75	31.36	29.96
0.95800	35.80	29.65	28.41	0.95516	37.80	31.40	30.00
0.95794	35.85	29.70	28.45	0.95509	37.85	31.45	30.04
0.95787	35.90	29.74	28.49	0.95502	37.90	31.49	30.08
0.95780	35.95	29.79	28.53	0.95494	37.95	31.54	30.12
0.95773	36.00	29.83	28.57	0.95487	38.00	31.58	30.16
0.95766	36.05	29.87	28.61	0.95480	38.05	31.63	30.20
0.95759	36.10	29.92	28.65	0.95472	38.10	31.67	30.24
0.95752	36.15	29.96	28.69	0.95465	38.15	31.72	30.28
0.95745	36.20	30.00	28.73	0.95457	38.20	31.76	30.32
0.95738	36.25	30.05	28.77	0.95450	38.25	31.81	30.36
0.95731	36.30	30.09	28.81	0.95442	38.30	31.85	30.40
0.95724	36.35	30.13	28.84	0.95435	38.35	31.90	30.44
0.95717	36.40	30.17	28.88	0.95427	38.40	31.94	30.48
0.95710	36.45	30.22	28.92	0.95420	38.45	31.99	30.52
0.95703	36.50	30.26	28.96	0.95413	38.50	32.03	30.56
0.95695	36.55	30.30	29.00	0.95405	38.55	32.07	30.60
0.95688	36.60	30.35	29.04	0.95398	38.60	32.12	30.64
0.95681	36.65	30.39	29.08	0.95390	38.65	32.16	30.68
0.95674	36.70	30.44	29.12	0.95383	38.70	32.20	30.72
0.95667	36.75	30.48	29.16	0.95375	38.75	32.25	30.76
0.95660	36.80	30.52	29.20	0.95368	38.80	32.29	30.79
0.95653	36.85	30.57	29.24	0.95360	38.85	32.33	30.83
0.95646	36.90	30.61	29.29	0.95353	38.90	32.37	30.87
0.95639	36.95	30.66	29.32	0.95345	38.95	32.42	30.91
0.95632	37.00	30.70	29.36	0.95338	39.00	32.46	30.95
0.95625	37.05	30.74	29.40	0.95330	39.05	32.50	30.99
0.95618	37.10	30.79	29.44	0.95323	39.10	32.55	31.03
0.95610	37.15	30.83	29.48	0.95315	39.15	32.59	31.07
0.95603	37.20	30.88	29.52	0.95307	39.20	32.64	31.11
0.95596	37.25	30.92	29.56	0.95300	39.25	32.68	31.14
0.95589	37.30	30.96	29.60	0.95292	39.30	32.72	31.18
0.95581	37.35	31.01	29.64	0.95284	39.35	32.77	31.22
0.95574	37.40	31.05	29.68	0.95277	39.40	32.81	31.26
0.95567	37.45	31.10	29.72	0.95269	39.45	32.86	31.30

PERCENTAGE OF ALCOHOL, BY WEIGHT, ETC.—Continued.

Specific gravity at 60°F.	Alcohol.			Specific gravity at 60°F.	Alcohol.		
	Per cent. by volume.	Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by volume.	Per cent. by weight.	Grams per 100 cubic centimeters.
0.95262	39.50	32.90	31.34	0.96329	31.75	26.16	25.20
0.95254	39.55	32.95	31.38	0.96323	31.80	26.20	25.24
0.95246	39.60	32.99	31.42	0.96316	31.85	26.24	25.28
0.95239	39.65	33.04	31.46	0.96310	31.90	26.28	25.32
0.95231	39.70	33.08	31.50	0.96304	31.95	26.33	25.36
0.95223	39.75	33.13	31.54	0.96298	32.00	26.37	25.40
0.95216	39.80	33.17	31.58	0.96292	32.05	26.41	25.44
0.95208	39.85	33.22	31.62	0.96285	32.10	26.46	25.48
0.95200	39.90	33.27	31.66	0.96279	32.15	26.50	25.52
0.95193	39.95	33.31	31.70	0.96273	32.20	26.54	25.56
0.95185	40.00	33.35	31.74	0.96267	32.25	26.59	25.60
0.95177	40.05	33.39	31.78	0.96260	32.30	26.63	25.64
0.95169	40.10	33.44	31.82	0.96254	32.35	26.67	25.68
0.95161	40.15	33.48	31.86	0.96248	32.40	26.71	25.71
0.95154	40.20	33.53	31.90	0.96241	32.45	26.76	25.75
0.95146	40.25	33.57	31.94	0.96235	32.50	26.80	25.79
0.95138	40.30	33.61	31.98	0.96229	32.55	26.84	25.83
0.95130	40.35	33.66	32.02	0.96222	32.60	26.89	25.87
0.95122	40.40	33.70	32.06	0.96216	32.65	26.93	25.91
0.95114	40.45	33.75	32.10	0.96210	32.70	26.97	25.95
0.95107	40.50	33.79	32.14	0.96204	32.75	27.02	25.99
0.95099	40.55	33.84	32.18	0.96197	32.80	27.06	26.03
0.95091	40.60	33.88	32.22	0.96191	32.85	27.10	26.07
0.95083	40.65	33.93	32.26	0.96185	32.90	27.14	26.11
0.95075	40.70	33.97	32.30	0.96178	32.95	27.19	26.15
0.95067	40.75	34.02	32.34	0.96172	33.00	27.23	26.19
0.95059	40.80	34.06	32.38	0.96166	33.05	27.27	26.23
0.95052	40.85	34.11	32.42	0.96159	33.10	27.32	26.27
0.95044	40.90	34.15	32.46	0.96153	33.15	27.36	26.31
0.45036	40.95	34.20	32.50	0.96146	33.20	27.40	26.35
0.95028	41.00	34.24	32.54	0.96140	33.25	27.45	26.39
0.95020	41.05	34.28	32.58	0.96133	33.30	27.49	26.43
0.95012	41.10	34.33	32.62	0.96127	33.35	27.53	26.47
0.95004	41.15	34.37	32.66	0.96120	33.40	27.57	26.51
0.94996	41.20	34.42	32.70	0.96114	33.45	27.62	26.55
0.96360	31.50	25.94	25.00	0.96108	33.50	27.66	26.59
0.96353	31.55	25.98	25.04	0.96101	33.55	27.70	26.63
0.96347	31.60	26.03	25.08	0.96095	33.60	27.75	26.67
0.96341	31.65	26.07	25.12	0.96088	33.65	27.79	26.71
0.96335	30.70	26.11	25.16	0.96082	33.70	27.83	26.75

PERCENTAGE OF ALCOHOL, BY WEIGHT, ETC.—*Continued.*

Specific gravity at 59°F.	Alcohol.			Specific gravity at 59°F.	Alcohol.		
	Per cent. by volume.	Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by volume.	Per cent. by weight.	Grams per 100 cubic centimeters.
0.96075	33.75	27.88	26.79	0.95807	35.75	29.61	28.37
0.96069	33.80	27.92	26.82	0.95800	35.80	29.65	28.41
0.96062	33.85	27.96	26.86	0.95794	35.85	29.70	28.45
0.96056	33.90	28.00	26.90	0.95787	35.90	29.74	28.49
0.96049	33.95	28.05	26.94	0.95780	35.95	29.79	28.53
0.96043	34.00	28.09	26.98	0.95773	36.00	29.83	28.57
0.96036	34.05	28.13	27.02	0.95766	36.05	29.87	28.61
0.96030	34.10	28.18	27.06	0.95759	36.10	29.92	28.65
0.96023	34.15	28.22	27.10	0.95752	36.15	29.96	28.69
0.96016	34.20	28.26	27.14	0.95745	36.20	30.00	28.73
0.96010	34.25	28.31	27.18	0.95738	36.25	30.05	28.77
0.96003	34.30	28.35	27.22	0.95731	36.30	30.09	28.81
0.95996	34.35	28.39	27.26	0.95724	36.35	30.13	28.84
0.95990	34.40	28.43	27.30	0.95717	36.40	30.17	28.88
0.95983	34.45	28.48	27.34	0.95710	36.45	30.22	28.92
0.95977	34.50	28.52	27.38	0.95703	36.50	30.26	28.96
0.95970	34.55	28.56	27.42	0.95695	36.55	30.30	29.00
0.95963	34.60	28.61	27.46	0.95688	36.60	30.35	29.04
0.95957	34.65	28.65	27.50	0.95681	36.65	30.39	29.08
0.95950	34.70	28.70	27.54	0.95674	36.70	30.44	29.12
0.95943	34.75	28.74	27.58	0.95667	36.75	30.48	29.16
0.95937	34.80	28.78	27.62	0.95660	36.80	30.52	29.20
0.95930	34.85	28.83	27.66	0.95653	36.85	30.57	29.24
0.95923	34.90	28.87	27.70	0.95646	36.90	30.61	29.29
0.95917	34.95	28.92	27.74	0.95639	36.95	30.66	29.32
0.95910	35.00	28.96	27.78	0.95632	37.00	30.70	29.36
0.95903	35.05	29.00	27.82	0.95625	37.05	30.74	29.40
0.95896	35.10	29.05	27.86	0.95618	37.10	30.79	29.44
0.95889	35.15	29.09	27.90	0.95610	37.15	30.83	29.48
0.95883	35.20	29.13	27.94	0.95603	37.20	30.88	29.52
0.95876	35.25	29.18	27.98	0.95596	37.25	30.92	29.56
0.95869	35.30	29.22	28.02	0.95589	37.30	30.96	29.60
0.95862	35.35	29.26	28.05	0.95581	37.35	31.01	29.64
0.95855	35.40	29.30	28.09	0.95574	37.40	31.05	29.68
0.95848	35.45	29.35	28.13	0.95567	37.45	31.10	29.72
0.95842	35.50	29.40	28.17	0.95560	37.50	31.14	29.76
0.95835	35.55	29.43	28.21	0.95552	37.55	31.18	29.80
0.95828	35.60	29.48	28.25	0.95545	37.60	31.23	29.84
0.95821	35.65	29.52	28.30	0.95538	37.65	31.27	29.88
0.95814	35.70	27.57	28.33	0.95531	37.70	31.32	29.92

PERCENTAGE OF ALCOHOL, BY WEIGHT, ETC.—*Continued.*

Specific gravity at 60°F.	Alcohol.			Specific gravity at 60°F.	Alcohol.		
	Per cent. by volume.	Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by volume.	Per cent. by weight.	Grams per 100 cubic centimeters.
0.95523	37.75	31.36	29.96	0.95262	39.50	32.90	31.34
0.95516	37.80	31.40	30.00	0.95254	39.55	32.95	31.38
0.95509	37.85	31.45	30.04	0.95246	39.60	32.99	31.42
0.95502	37.90	31.49	30.08	0.95239	39.65	33.04	31.46
0.95494	37.95	31.54	30.12	0.95231	39.70	33.08	31.50
0.95487	38.00	31.58	30.16	0.95223	39.75	33.13	31.54
0.95480	38.05	31.63	30.20	0.95216	39.80	33.17	31.58
0.95472	38.10	31.67	30.24	0.95208	39.85	33.22	31.62
0.95465	38.15	31.72	30.28	0.95200	39.90	33.27	31.66
0.95457	38.20	31.76	30.32	0.95193	39.95	33.31	31.70
0.95450	38.25	31.81	30.36	0.95185	40.00	33.35	31.74
0.95442	38.30	31.85	30.40	0.95177	40.05	33.39	31.78
0.95435	38.35	31.90	30.44	0.95169	40.10	33.44	31.82
0.95427	38.40	31.94	30.48	0.95161	40.15	33.48	31.86
0.95420	38.45	31.99	30.52	0.95154	40.20	33.53	31.90
0.95413	38.50	32.03	30.56	0.95146	40.25	33.57	31.94
0.95405	38.55	32.07	30.60	0.95138	40.30	33.61	31.98
0.95398	38.60	32.12	30.64	0.95130	40.35	33.66	32.02
0.95390	38.65	32.16	30.68	0.95122	40.40	33.70	32.06
0.95383	38.70	32.20	30.72	0.95114	40.45	33.75	32.10
0.95375	38.75	32.25	30.76	0.95107	40.50	33.79	32.14
0.95368	38.80	32.29	30.79	0.95099	40.55	33.84	32.18
0.95360	38.85	32.33	30.83	0.95091	40.60	33.88	32.22
0.95353	38.90	32.37	30.87	0.95083	40.65	33.93	32.26
0.95345	38.95	32.42	30.91	0.95075	40.70	33.97	32.30
0.95338	39.00	32.46	30.95	0.95067	40.75	34.02	32.34
0.95330	39.05	32.50	30.99	0.95059	40.80	34.06	32.38
0.95323	39.10	32.55	31.03	0.95052	40.85	34.11	32.42
0.95315	39.15	32.59	31.07	0.95044	40.90	34.15	32.46
0.95307	39.20	32.64	31.11	0.95036	40.95	34.20	32.50
0.95300	39.25	32.68	31.14	0.95028	41.00	34.24	32.54
0.95292	39.30	32.72	31.18	0.95020	41.05	34.28	32.58
0.95284	39.35	32.77	31.22	0.95012	41.10	34.33	32.62
0.95277	39.40	32.81	31.26	0.95004	41.15	34.37	32.66
0.95269	39.45	32.86	31.30	0.94996	41.20	34.42	32.70

662. Determination of Percentage of Alcohol by Means of Vapor Temperature.—The temperature of a mixture of alcohol and water vapors is less than that of water alone and the depression

is proportional to the quantity of alcohol present. This principle is utilized in the construction of the ebullioscope or ebulliometer. In this apparatus the temperature of pure boiling water vapor is determined by a preliminary experiment. This point must be frequently revised in order to correct it for variations in barometric pressure. The water is withdrawn from the boiler of the apparatus, the same volume of a wine or beer placed therein, and the vapor temperature again determined. By comparing the boiling point of the wine, with a scale calibrated for different percentages of alcohol, the quantity of spirit present is determined. When water vapor is at 100° a wine having eight per cent. of alcohol gives a vapor at $93^{\circ}.8$. The presence of extractive matters in the sample, which tend to raise its boiling point, is neglected in the calculation of results.

663. Improved Ebullioscope.—The principle mentioned in the above paragraph may be applied with a considerable degree of accuracy, by using the improved ebullioscope described below.⁸²

The apparatus consists of a glass flask F, shaped somewhat like an Erlenmeyer, closed at the top with a rubber stopper carrying a central aperture for the insertion of the delicate thermometer AB, and a lateral smaller aperture for connecting the interior of the flask with the condenser D. The return of the condensed vapors from D is effected through the tube entering the flask F in such a manner as to deliver the condensed liquid beneath the surface of the liquid in F as shown in the figure. The flask F contains pieces of scrap platinum or pumice stone to prevent bumping and secure an even ebullition. The flask F rests upon a disk of asbestos, perforated in such a way as to have the opening fully covered by the bottom of the flask. To protect F against the influence of air currents it is enclosed in the glass cylinder E resting on the asbestos disk below and closed with a detachable soft rubber cover at the top. The temperature between the cylinder E and the flask F is measured by the thermometer C and the flame of the lamp G should be so adjusted as to bring the temperature between the flask F and the

⁸² Wiley, *Journal of the American Chemical Society*, 1896, **18** : 1063.

cylinder E, to about 90° at the time of reading the thermometer B. The bulb of the thermometer B may be protected by a thin glass tube carrying distilled water, so adjusted as to prevent the escape of the watery vapor into F. The thermometer B is such

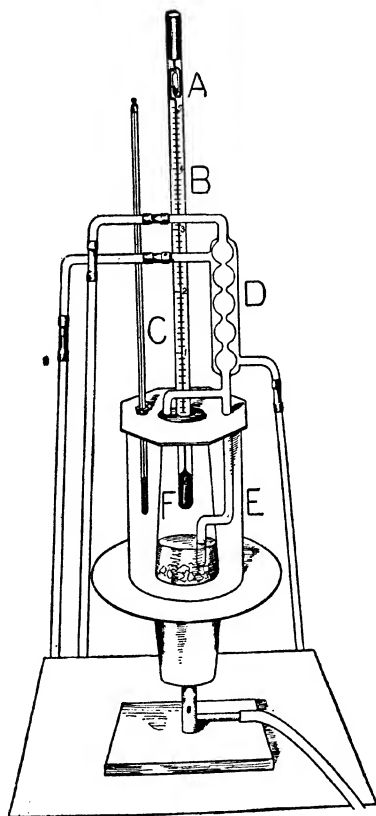


Fig. 117.—Improved Ebullioscope.

as is used for determining molecular weights by the cryoscopic method. It has a cistern at A which holds any excess of mercury not needed in adjusting the thermometer for any required temperature.

A second apparatus, exactly similar to the one described, is

conveniently used for measuring the changes in barometric pressure during the process of the analysis. The temperature of the vapor of boiling water having been first determined, the beer or wine is placed in F, and the temperature of the vapor of the boiling liquid determined after the temperature of the air layer between E and F reaches about 90°, measured on the thermometer C. By using alcoholic mixtures of known strength the depression for each changing per cent. of alcohol is determined for each system of apparatus employed, and this having once been done, the percentage of alcohol in any unknown liquid is at once determined by inspecting the thermometer, the bulb of which is immersed in the vapor from the boiling liquid. In the apparatus figured, a depression of 0°.8 is equivalent to one per cent. of alcohol by volume. Full directions for the manipulation of the apparatus may be found in the paper cited above.

664. Total Fixed Matters.—The residue left on evaporating a fermented beverage to dryness is commonly known as extractive matter, or simply extract. It is composed chiefly of unfermented carbohydrates, organic acids, nitrogenous bodies, glycerol and mineral substances. Hydrochloric and sulfuric acids may also be found therein. If any non-volatile preservatives have been used in the sample, such as borax, salicylates and the like, these will also be found in the solid residue. The bodies which escape are water, alcohols, ethers and essential oils. The character of the residue left by wines and beers is evidently different. In each case it should contain typical components which aid in judging of the purity of the sample. For instance, in beers the substitution for malt of carbohydrate bodies comparatively free from proteins, produces a beer containing a deficiency of nitrogenous bodies. Pure malt beer will rarely have less than one-half of a per cent. of proteins, while beer made largely of glucose, rice or hominy grits, will have a much smaller quantity. First will be described below the methods of determining the fixed residue left on evaporation, and thereafter the processes for ascertaining its leading components.

665. Methods of the Official Chemists.—Two methods are in

use by the official chemists for determining the non-volatile bodies in wines.⁸³ They are as follows:

(a) *From Specific Gravity of Dealcoholized Wine.*—Preliminary to its exact determination the extract should be calculated by the formula: $sp. = 1 + a - b$

in which "*sp.*" is the specific gravity of the dealcoholized wine, "*a*" the specific gravity of the wine, and "*b*" the specific gravity of the alcoholic distillate obtained in the estimation of alcohol.

Illustration:—A sample of wine examined gave the following data:

Specific gravity of wine (a).....	1.0402
Specific gravity of alcoholic distillate (b).....	0.9857
Difference (a—b).....	0.0545
Specific gravity of dealcoholized wine (1 + a—b).....	1.0545
Extract (grams per 100 cubic centimeters).....	14.12

The extract equivalent of "*sp.*" is obtained from the appended table.

(b) *By Evaporation.*—(1) *In Dry Wines.*—Having an extract content of less than three grams per 100 cubic centimeters. Evaporate 50 cubic centimeters of the sample on the water bath to a sirupy consistency in a flat-bottom platinum dish approximately 85 millimeters in diameter and of about 75 cubic centimeters capacity. Heat the residue for two and one-half hours in a drying oven at the temperature of boiling water and weigh. The sugar-free extract is found by deducting the weight of sugar in excess of 0.1 gram per 100 cubic centimeters from the total residue. In the case of plastered wines, the potassium sulfate in excess of 0.1 gram is also deducted.

(2) *In Sweet Wines.*—When the extract content is between three and six grams per 100 cubic centimeters treat 25 cubic centimeters of the sample as described under dry wines. When the extract exceeds six grams per 100 cubic centimeters, however, the result obtained under (a) is accepted, and no gravimetric determination is attempted. This is because of the serious error connected with drying levulose at high temperature. (The table referred to under (a) was obtained by drying at 75° in vacuo.)

⁸³ Bureau of Chemistry, U. S. Department of Agriculture, Bulletin 107 (revised), 1912: 84.

EXTRACT IN DEALCOHOLIZED WINE

[According to Windisch.]

Specific gravity	Extract	Specific gravity	Extract	Specific gravity	Extract	Specific gravity	Extract
1.0000	0.00	1.0040	1.03	1.0080	2.07	1.0120	3.10
1.0001	0.03	1.0041	1.05	1.0081	2.09	1.0121	3.12
1.0002	0.05	1.0042	1.08	1.0082	2.12	1.0122	3.15
1.0003	0.08	1.0043	1.11	1.0083	2.14	1.0123	3.18
1.0004	0.10	1.0044	1.13	1.0084	2.17	1.0124	3.20
1.0005	0.13	1.0045	1.16	1.0085	2.19	1.0125	3.23
1.0006	0.15	1.0046	1.18	1.0086	2.22	1.0126	3.26
1.0007	0.18	1.0047	1.21	1.0087	2.25	1.0127	3.28
1.0008	0.20	1.0048	1.24	1.0088	2.27	1.0128	3.31
1.0009	0.23	1.0049	1.26	1.0089	2.30	1.0129	3.33
1.0010	0.26	1.0050	1.29	1.0090	2.32	1.0130	3.36
1.0011	0.28	1.0051	1.32	1.0091	2.35	1.0131	3.38
1.0012	0.31	1.0052	1.34	1.0092	2.38	1.0132	3.41
1.0013	0.34	1.0053	1.37	1.0093	2.40	1.0133	3.43
1.0014	0.36	1.0054	1.39	1.0094	2.43	1.0134	3.46
1.0015	0.39	1.0055	1.42	1.0095	2.45	1.0135	3.49
1.0016	0.41	1.0056	1.45	1.0096	2.48	1.0136	3.51
1.0017	0.44	1.0057	1.47	1.0097	2.50	1.0137	3.54
1.0018	0.46	1.0058	1.50	1.0098	2.53	1.0138	3.56
1.0019	0.49	1.0059	1.52	1.0099	2.56	1.0139	3.59
1.0020	0.52	1.0060	1.55	1.0100	2.58	1.0140	3.62
1.0021	0.54	1.0061	1.57	1.0101	2.61	1.0141	3.64
1.0022	0.57	1.0062	1.60	1.0102	2.63	1.0142	3.67
1.0023	0.59	1.0063	1.63	1.0103	2.66	1.0143	3.69
1.0024	0.62	1.0064	1.65	1.0104	2.69	1.0144	3.72
1.0025	0.64	1.0065	1.68	1.0105	2.71	1.0145	3.75
1.0026	0.67	1.0066	1.70	1.0106	2.74	1.0146	3.77
1.0027	0.69	1.0067	1.73	1.0107	2.76	1.0147	3.80
1.0028	0.72	1.0068	1.76	1.0108	2.79	1.0148	3.82
1.0029	0.75	1.0069	1.78	1.0109	2.82	1.0149	3.85
1.0030	0.77	1.0070	1.81	1.0110	2.84	1.0150	3.87
1.0031	0.80	1.0071	1.83	1.0111	2.87	1.0151	3.90
1.0032	0.82	1.0072	1.86	1.0112	2.89	1.0152	3.93
1.0033	0.85	1.0073	1.88	1.0113	2.92	1.0153	3.95
1.0034	0.87	1.0074	1.91	1.0114	2.94	1.0154	3.98
1.0035	0.90	1.0075	1.94	1.0115	2.97	1.0155	4.00
1.0036	0.93	1.0076	1.96	1.0116	3.00	1.0156	4.03
1.0037	0.95	1.0077	1.99	1.0117	3.02	1.0157	4.06
1.0038	0.98	1.0078	2.01	1.0118	3.05	1.0158	4.08
1.0039	1.00	1.0079	2.04	1.0119	3.07	1.0159	4.11

EXTRACT IN DEALCOHOLIZED WINE.—*Continued.*

Specific gravity	Extract	Specific gravity	Extract	Specific gravity	Extract	Specific gravity	Extract
1.0160	4.13	1.0205	5.30	1.0250	6.46	1.0295	7.63
1.0161	4.16	1.0206	5.32	1.0251	6.49	1.0296	7.65
1.0162	4.19	1.0207	5.35	1.0252	6.51	1.0297	7.68
1.0163	4.21	1.0208	5.38	1.0253	6.54	1.0298	7.70
1.0164	4.24	1.0209	5.40	1.0254	6.56	1.0299	7.73
1.0165	4.26	1.0210	5.43	1.0255	6.59	1.0300	7.76
1.0166	4.29	1.0211	5.45	1.0256	6.62	1.0301	7.78
1.0167	4.31	1.0212	5.48	1.0257	6.64	1.0302	7.81
1.0168	4.34	1.0213	5.51	1.0258	6.67	1.0303	7.83
1.0169	4.37	1.0214	5.53	1.0259	6.70	1.0304	7.86
1.0170	4.39	1.0215	5.56	1.0260	6.72	1.0305	7.89
1.0171	4.42	1.0216	5.58	1.0261	6.75	1.0306	7.91
1.0172	4.44	1.0217	5.61	1.0262	6.77	1.0307	7.94
1.0173	4.47	1.0218	5.64	1.0263	6.80	1.0308	7.97
1.0174	4.50	1.0219	5.66	1.0264	6.82	1.0309	7.99
1.0175	4.52	1.0220	5.69	1.0265	6.85	1.0310	8.02
1.0176	4.55	1.0221	5.71	1.0266	6.88	1.0311	8.04
1.0177	4.57	1.0222	5.74	1.0267	6.90	1.0312	8.07
1.0178	4.60	1.0223	5.77	1.0268	6.93	1.0313	8.09
1.0179	4.63	1.0224	5.79	1.0269	6.95	1.0314	8.12
1.0180	4.65	1.0225	5.82	1.0270	6.98	1.0315	8.14
1.0181	4.68	1.0226	5.84	1.0271	7.01	1.0316	8.17
1.0182	4.70	1.0227	5.87	1.0272	7.03	1.0317	8.20
1.0183	4.73	1.0228	5.89	1.0273	7.06	1.0318	8.22
1.0184	4.75	1.0229	5.92	1.0274	7.08	1.0319	8.25
1.0185	4.78	1.0230	5.94	1.0275	7.11	1.0320	8.27
1.0186	4.81	1.0231	5.97	1.0276	7.13	1.0321	8.30
1.0187	4.83	1.0232	6.00	1.0277	7.16	1.0322	8.33
1.0188	4.86	1.0233	6.02	1.0278	7.19	1.0323	8.35
1.0189	4.88	1.0234	6.05	1.0279	7.21	1.0324	8.38
1.0190	4.91	1.0235	6.07	1.0280	7.24	1.0325	8.40
1.0191	4.94	1.0236	6.10	1.0281	7.26	1.0326	8.43
1.0192	4.96	1.0237	6.12	1.0282	7.29	1.0327	8.46
1.0193	4.99	1.0238	6.15	1.0283	7.32	1.0328	8.48
1.0194	5.01	1.0239	6.18	1.0284	7.34	1.0329	8.51
1.0195	5.04	1.0240	6.20	1.0285	7.37	1.0330	8.53
1.0196	5.06	1.0241	6.23	1.0286	7.39	1.0331	8.56
1.0197	5.09	1.0242	6.25	1.0287	7.42	1.0332	8.59
1.0198	5.11	1.0243	6.28	1.0288	7.45	1.0333	8.61
1.0199	5.14	1.0244	6.31	1.0289	7.47	1.0334	8.64
1.0200	5.17	1.0245	6.33	1.0290	7.50	1.0335	8.66
1.0201	5.19	1.0246	6.36	1.0291	7.52	1.0336	8.69
1.0202	5.22	1.0247	6.38	1.0292	7.55	0.0337	8.72
1.0203	5.25	1.0248	6.41	1.0293	7.58	0.0338	8.74
1.0204	5.27	1.0249	6.44	1.0294	7.60	1.0339	8.77

EXTRACT IN DEALCOHOLIZED WINE.—*Continued.*

Specific gravity.	Extract.	Specific gravity.	Extract.	Specific gravity.	Extract.	Specific gravity.	Extract.
1.0340	8.79	1.0385	9.96	1.0430	11.13	1.0475	12.30
1.0341	8.82	1.0386	9.99	1.0431	11.15	1.0476	12.32
1.0342	8.85	1.0387	10.01	1.0432	11.18	1.0477	12.35
1.0343	8.87	1.0388	10.04	1.0433	11.21	1.0478	12.38
1.0344	8.90	1.0389	10.06	1.0434	11.23	1.0479	12.40
1.0345	8.92	1.0390	10.09	1.0435	11.26	1.0480	12.43
1.0346	8.95	1.0391	10.11	1.0436	11.28	1.0481	12.45
1.0347	8.97	1.0392	10.14	1.0437	11.31	1.0482	12.48
1.0348	9.00	1.0393	10.17	1.0438	11.34	1.0483	12.51
1.0349	9.03	1.0394	10.19	1.0439	11.36	1.0484	12.53
1.0350	9.05	1.0395	10.22	1.0440	11.39	1.0485	12.56
1.0351	9.08	1.0396	10.25	1.0441	11.42	1.0486	12.58
1.0352	9.10	1.0397	10.27	1.0442	11.44	1.0487	12.61
1.0353	9.13	1.0398	10.30	1.0443	11.47	1.0488	12.64
1.0354	9.16	1.0399	10.32	1.0444	11.49	1.0489	12.66
1.0355	9.18	1.0400	10.35	1.0445	11.52	1.0490	12.69
1.0356	9.21	1.0401	10.37	1.0446	11.55	1.0491	12.71
1.0357	9.23	1.0402	10.40	1.0447	11.57	1.0492	12.74
1.0358	9.26	1.0403	10.43	1.0448	11.60	1.0493	12.77
1.0359	9.29	1.0404	10.45	1.0449	11.62	1.0494	12.79
1.0360	9.31	1.0405	10.48	1.0450	11.65	1.0495	12.82
1.0361	9.34	1.0406	10.51	1.0451	11.68	1.0496	12.84
1.0362	9.36	1.0407	10.53	1.0452	11.70	1.0497	12.87
1.0363	9.39	1.0408	10.56	1.0453	11.73	1.0498	12.90
1.0364	9.42	1.0409	10.58	1.0454	11.75	1.0499	12.92
1.0365	9.44	1.0410	10.61	1.0455	11.78	1.0500	12.95
1.0366	9.47	1.0411	10.63	1.0456	11.81	1.0501	12.97
1.0367	9.49	1.0412	10.66	1.0457	11.83	1.0502	13.00
1.0368	9.52	1.0413	10.69	1.0458	11.86	1.0503	13.03
1.0369	9.55	1.0414	10.71	1.0459	11.88	1.0504	13.05
1.0370	9.57	1.0415	10.74	1.0460	11.91	1.0505	13.08
1.0371	9.60	1.0416	10.76	1.0461	11.94	1.0506	13.10
1.0372	9.62	1.0417	10.79	1.0462	11.96	1.0507	13.13
1.0373	9.65	1.0418	10.82	1.0463	11.99	1.0508	13.16
1.0374	9.68	1.0419	10.84	1.0464	12.01	1.0509	13.18
1.0375	9.70	1.0420	10.87	1.0465	12.04	1.0510	13.21
1.0376	9.73	1.0421	10.90	1.0466	12.06	1.0511	13.23
1.0377	9.75	1.0422	10.92	1.0467	12.09	1.0512	13.26
1.0378	9.78	1.0423	10.95	1.0468	12.12	1.0513	13.29
1.0379	9.80	1.0424	10.97	1.0469	12.14	1.0514	13.31
1.0380	9.83	1.0425	11.00	1.0470	12.17	1.0515	13.34
1.0381	9.86	1.0426	11.03	1.0471	12.19	1.0516	13.36
1.0382	9.88	1.0427	11.05	1.0472	12.22	1.0517	13.39
1.0383	9.91	1.0428	11.08	1.0473	12.25	1.0518	13.42
1.0384	9.93	1.0429	11.10	1.0474	12.27	1.0519	13.44

EXTRACT IN DEALCOHOLIZED WINE.—*Continued.*

Specific gravity.	Extract.	Specific gravity.	Extract.	Specific gravity.	Extract.	Specific gravity.	Extract.
1.0520	13.47	1.0565	14.64	1.0610	15.81	1.0655	16.99
1.0521	13.49	1.0566	14.67	1.0611	15.84	1.0656	17.01
1.0522	13.52	1.0567	14.69	1.0612	15.87	1.0657	17.04
1.0523	13.55	1.0568	14.72	1.0613	15.89	1.0658	17.07
1.0524	13.57	1.0569	14.74	1.0614	15.92	1.0659	17.09
1.0525	13.60	1.0570	14.77	1.0615	15.94	1.0660	17.12
1.0526	13.62	1.0571	14.80	1.0616	15.97	1.0661	17.14
1.0527	13.65	1.0572	14.82	1.0617	16.00	1.0662	17.17
1.0528	13.68	1.0573	14.85	1.0618	16.02	1.0663	17.20
1.0529	13.70	1.0574	14.87	1.0619	16.05	1.0664	17.22
1.0530	13.73	1.0575	14.90	1.0620	16.07	1.0665	17.25
1.0531	13.75	1.0576	14.93	1.0621	16.10	1.0666	17.27
1.0532	13.78	1.0577	14.95	1.0622	16.13	1.0667	17.30
1.0533	13.81	1.0578	14.98	1.0623	16.15	1.0668	17.33
1.0534	13.83	1.0579	15.00	1.0624	16.18	1.0669	17.35
1.0535	13.86	1.0580	15.03	1.0625	16.21	1.0670	17.38
1.0536	13.89	1.0581	15.06	1.0626	16.23	1.0671	17.41
1.0537	13.91	1.0582	15.08	1.0627	16.26	1.0672	17.43
1.0538	13.94	1.0583	15.11	1.0628	16.28	1.0673	17.46
1.0539	13.96	1.0584	15.14	1.0629	16.31	1.0674	17.48
1.0540	13.99	1.0585	15.16	1.0630	16.33	1.0675	17.51
1.0541	14.01	1.0586	15.19	1.0631	16.36	1.0676	17.54
1.0542	14.04	1.0587	15.22	1.0632	16.39	1.0677	17.56
1.0543	14.07	1.0588	15.24	1.0633	16.41	1.0678	17.59
1.0544	14.09	1.0589	15.27	1.0634	16.44	1.0679	17.62
1.0545	14.12	1.0590	15.29	1.0635	16.47	1.0680	17.64
1.0546	14.14	1.0591	15.32	1.0636	16.49	1.0681	17.67
1.0547	14.17	1.0592	15.35	1.0637	16.52	1.0682	17.69
1.0548	14.20	1.0593	15.37	1.0638	16.54	1.0683	17.72
1.0549	14.22	1.0594	15.40	1.0639	16.57	1.0684	17.75
1.0550	14.25	1.0595	15.42	1.0640	16.60	1.0685	17.77
1.0551	14.28	1.0596	15.45	1.0641	16.62	1.0686	17.80
1.0552	14.30	1.0597	15.48	1.0642	16.65	1.0687	17.83
1.0553	14.33	1.0598	15.50	1.0643	16.68	1.0688	17.85
1.0554	14.35	1.0599	15.53	1.0644	16.70	1.0689	17.88
1.0555	14.38	1.0600	15.55	1.0645	16.73	1.0690	17.90
1.0556	14.41	1.0601	15.58	1.0646	16.75	1.0691	17.93
1.0557	14.43	1.0602	15.61	1.0647	16.78	1.0692	17.95
1.0558	14.46	1.0603	15.63	1.0648	16.80	1.0693	17.98
1.0559	14.48	1.0604	15.66	1.0649	16.83	1.0694	18.01
1.0560	14.51	1.0605	15.68	1.0650	16.86	1.0695	18.03
1.0561	14.54	1.0606	15.71	1.0651	16.88	1.0696	18.06
1.0562	14.56	1.0607	15.74	1.0652	16.91	1.0697	18.08
1.0563	14.59	1.0608	15.76	1.0653	16.94	1.0698	18.11
1.0564	14.61	1.0609	15.79	1.0654	16.96	1.0699	18.14

EXTRACT IN DEALCOHOLIZED WINE.—*Continued.*

Specific gravity.	Extract.	Specific gravity.	Extract.	Specific gravity.	Extract.	Specific gravity.	Extract.
1.0700	18.16	1.0745	19.34	1.0790	20.52	1.0835	21.70
1.0701	18.19	1.0746	19.37	1.0791	20.55	1.0836	21.73
1.0702	18.22	1.0747	19.39	1.0792	20.57	1.0837	21.75
1.0703	18.24	1.0748	19.42	1.0793	20.60	1.0838	21.78
1.0704	18.27	1.0749	19.44	1.0794	20.62	1.0839	21.80
1.0705	18.30	1.0750	19.47	1.0795	20.65	1.0840	21.83
1.0706	18.32	1.0751	19.50	1.0796	20.68	1.0841	21.86
1.0707	18.35	1.0752	19.52	1.0797	20.70	1.0842	21.88
1.0708	18.37	1.0753	19.55	1.0798	20.73	1.0843	21.91
1.0709	18.40	1.0754	19.58	1.0799	20.75	1.0844	21.94
1.0710	18.43	1.0755	19.60	1.0800	20.78	1.0845	21.96
1.0711	18.45	1.0756	19.63	1.0801	20.81	1.0846	21.99
1.0712	18.48	1.0757	19.65	1.0802	20.83	1.0847	22.02
1.0713	18.50	1.0758	19.68	1.0803	20.86	1.0848	22.04
1.0714	18.53	1.0759	19.71	1.0804	20.89	1.0849	22.07
1.0715	18.56	1.0760	19.73	1.0805	20.91	1.0850	22.09
1.0716	18.58	1.0761	19.76	1.0806	20.94	1.0851	22.12
1.0717	18.61	1.0762	19.79	1.0807	20.96	1.0852	22.15
1.0718	18.63	1.0763	19.81	1.0808	20.99	1.0853	22.17
1.0719	18.66	1.0764	19.84	1.0809	21.02	1.0854	22.20
1.0720	18.69	1.0765	19.86	1.0810	21.04	1.0855	22.22
1.0721	18.71	1.0766	19.89	1.0811	21.07	1.0856	22.25
1.0722	18.74	1.0767	19.92	1.0812	21.10	1.0857	22.28
1.0723	18.76	1.0768	19.94	1.0813	21.12	1.0858	22.30
1.0724	18.79	1.0769	19.97	1.0814	21.15	1.0859	22.33
1.0725	18.82	1.0770	20.00	1.0815	21.17	1.0860	22.36
1.0726	18.84	1.0771	20.02	1.0816	21.20	1.0861	22.38
1.0727	18.87	1.0772	20.05	1.0817	21.23	1.0862	22.41
1.0728	18.90	1.0773	20.07	1.0818	21.25	1.0863	22.43
1.0729	18.92	1.0774	20.10	1.0819	21.28	1.0864	22.46
1.0730	18.95	1.0775	20.12	1.0820	21.31	1.0865	22.49
1.0731	18.97	1.0776	20.15	1.0821	21.33	1.0866	22.51
1.0732	19.00	1.0777	20.18	1.0822	21.36	1.0867	22.54
1.0733	19.03	1.0778	20.20	1.0823	21.38	1.0868	22.57
1.0734	19.05	1.0779	20.23	1.0824	21.41	1.0869	22.59
1.0735	19.08	1.0780	20.26	1.0825	21.44	1.0870	22.62
1.0736	19.10	1.0781	20.28	1.0826	21.46	1.0871	22.65
1.0737	19.13	1.0782	20.31	1.0827	21.49	1.0872	22.67
1.0738	19.16	1.0783	20.34	1.0828	21.52	1.0873	22.70
1.0739	19.18	1.0784	20.36	1.0829	21.54	1.0874	22.72
1.0740	19.21	1.0785	20.39	1.0830	21.57	1.0875	22.75
1.0741	19.23	1.0786	20.41	1.0831	21.59	1.0876	22.78
1.0742	19.26	1.0787	20.44	1.0832	21.62	1.0877	22.80
1.0743	19.29	1.0788	20.47	1.0833	21.65	1.0878	22.83
1.0744	19.31	1.0789	20.49	1.0834	21.67	1.0879	22.86

EXTRACT IN DEALCOHOLIZED WINE

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EXTRACT IN DEALCOHOLIZED WINE.—Continued.

Specific gravity.	Extract.	Specific gravity.	Extract.	Specific gravity.	Extract.	Specific gravity.	Extract.
1.0880	22.88	1.0925	24.07	1.0970	25.25	1.1015	26.43
1.0881	22.91	1.0926	24.09	1.0971	25.28	1.1016	26.46
1.0882	22.93	1.0927	24.12	1.0972	25.30	1.1017	26.49
1.0883	22.96	1.0928	24.14	1.0973	25.33	1.1018	26.51
1.0884	22.99	1.0929	24.17	1.0974	25.36	1.1019	26.54
1.0885	23.01	1.0930	24.20	1.0975	25.38	1.1020	26.56
1.0886	23.04	1.0931	24.22	1.0976	25.41	1.1021	26.59
1.0887	23.07	1.0932	24.25	1.0977	25.43	1.1022	26.62
1.0888	23.09	1.0933	24.27	1.0978	25.46	1.1023	26.64
1.0889	23.12	1.0934	24.30	1.0979	25.49	1.1024	26.67
1.0890	23.14	1.0935	24.33	1.0980	25.51	1.1025	26.70
1.0891	23.17	1.0936	24.35	1.0981	25.54	1.1026	26.72
1.0892	23.20	1.0937	24.38	1.0982	25.56	1.1027	26.75
1.0893	23.22	1.0938	24.41	1.0983	25.59	1.1028	26.78
1.0894	23.25	1.0939	24.43	1.0984	25.62	1.1029	26.80
1.0895	23.28	1.0940	24.46	1.0985	25.64	1.1030	26.83
1.0896	23.30	1.0941	24.49	1.0986	25.67	1.1031	26.85
1.0897	23.33	1.0942	24.51	1.0987	25.70	1.1032	26.88
1.0898	23.35	1.0943	24.54	1.0988	25.72	1.1033	26.91
1.0899	23.38	1.0944	24.57	1.0989	25.75	1.1034	26.93
1.0900	23.41	1.0945	24.59	1.0990	25.78	1.1035	26.96
1.0901	23.43	1.0946	24.62	1.0991	25.80	1.1036	26.99
1.0902	23.46	1.0947	24.64	1.0992	25.83	1.1037	27.01
1.0903	23.49	1.0948	24.67	1.0993	25.85	1.1038	27.04
1.0904	23.51	1.0949	24.70	1.0994	25.88	1.1039	27.07
1.0905	23.54	1.0950	24.72	1.0995	25.91	1.1040	27.09
1.0906	23.57	1.0951	24.75	1.0996	25.93	1.1041	27.12
1.0907	23.59	1.0952	24.78	1.0997	25.96	1.1042	27.15
1.0908	23.62	1.0953	24.80	1.0998	25.99	1.1043	27.17
1.0909	23.65	1.0954	24.83	1.0999	26.01	1.1044	27.20
1.0910	23.67	1.0955	24.85	1.1000	26.04	1.1045	27.22
1.0911	23.70	1.0956	24.88	1.1001	26.06	1.1046	27.25
1.0912	23.72	1.0957	24.91	1.1002	26.09	1.1047	27.27
1.0913	23.75	1.0958	24.93	1.1003	26.12	1.1048	27.30
1.0914	23.77	1.0959	24.96	1.1004	26.14	1.1049	27.33
1.0915	23.80	1.0960	24.99	1.1005	26.17	1.1050	27.35
1.0916	23.83	1.0961	25.01	1.1006	26.20	1.1051	27.38
1.0917	23.85	1.0962	25.04	1.1007	26.22	1.1052	27.41
1.0918	23.88	1.0963	25.07	1.1008	26.25	1.1053	27.43
1.0919	23.91	1.0964	25.09	1.1009	26.27	1.1054	27.46
1.0920	23.93	1.0965	25.12	1.1010	26.30	1.1055	27.49
1.0921	23.96	1.0966	25.14	1.1011	26.33	1.1056	27.51
1.0922	23.99	1.0967	25.17	1.1012	26.35	1.1057	27.54
1.0923	24.01	1.0968	25.20	1.1013	26.38	1.1058	27.57
1.0924	24.04	1.0969	25.22	1.1014	26.41	1.1059	27.59

EXTRACT IN DEALCOHOLIZED WINE.—*Continued.*

Specific gravity.	Extract.	Specific gravity.	Extract.	Specific gravity.	Extract.	Specific gravity.	Extract.
1.1060	27.62	1.1085	28.28	1.1110	28.94	1.1135	29.60
1.1061	27.65	1.1086	28.30	1.1111	28.96	1.1136	29.62
1.1062	27.67	1.1087	28.33	1.1112	28.99	1.1137	29.65
1.1063	27.70	1.1088	28.36	1.1113	29.02	1.1138	29.68
1.1064	27.72	1.1089	28.38	1.1114	29.04	1.1139	29.70
1.1065	27.75	1.1090	28.41	1.1115	29.07	1.1140	29.73
1.1066	27.78	1.1091	28.43	1.1116	29.09	1.1141	29.76
1.1067	27.80	1.1092	28.46	1.1117	29.12	1.1142	29.78
1.1068	27.83	1.1093	28.49	1.1118	29.15	1.1143	29.81
1.1069	27.86	1.1094	28.51	1.1119	29.17	1.1144	29.83
1.1070	27.88	1.1095	28.54	1.1120	29.20	1.1145	29.86
1.1071	27.90	1.1096	28.57	1.1121	29.23	1.1146	29.89
1.1072	27.93	1.1097	28.59	1.1122	29.25	1.1147	29.91
1.1073	27.96	1.1098	28.62	1.1123	29.28	1.1148	29.94
1.1074	27.99	1.1099	28.65	1.1124	29.31	1.1149	29.96
1.1075	28.01	1.1100	28.67	1.1125	29.33	1.1150	29.99
1.1076	28.04	1.1101	28.70	1.1126	29.36	1.1151	30.02
1.1077	28.07	1.1102	28.73	1.1127	29.39	1.1152	30.04
1.1078	28.09	1.1103	28.75	1.1128	29.41	1.1153	30.07
1.1079	28.12	1.1104	28.78	1.1129	29.44	1.1154	30.10
1.1080	28.15	1.1105	28.81	1.1130	29.47	1.1155	30.13
1.1081	28.17	1.1106	28.83	1.1131	29.49	1.1156	30.15
1.1082	28.20	1.1107	28.86	1.1132	29.52	1.1157	30.18
1.1083	28.22	1.1108	28.88	1.1133	29.54	1.1158	30.21
1.1084	28.25	1.1109	28.91	1.1134	29.57	1.1159	30.23

Extract of Beers.—The following methods are used by the Association of Official Agricultural Chemists for the determination of extract in beer:⁸⁴

(a) *Method I.*—Evaporate 25 cubic centimeters of the beer in a tared platinum dish to constant weight in a water oven at 80°.

(b) *Optional Method II.*—Calculate according to formula $sp = g + (1-a)$, in which sp is the specific gravity of the dealcoholized beer, g the specific gravity of the beer, and a the specific gravity of the distillate obtained in the determination of alcohol, and determine value of sp from appended table of Schultz and Ostermann.

(c) *Optional Method III.*—Make immersion refractometer reading of dealcoholized beer and calculate extract in grams per 100 cubic centimeters.

⁸⁴ Bureau of Chemistry, U. S. Department of Agriculture, Bulletin 187, (revised), 1912: 90 and 209.

EXTRACT IN BEER WORT

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EXTRACT IN BEER WORT.⁸⁵

[According to Schultz and Ostermann.]

Specific gravity at 15°C.	Extract.		Specific gravity at 15°C.	Extract.		Specific gravity at 15°C.	Extract.	
	Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by weight.	Grams per 100 cubic centimeters.
1.0000	0.00	0.00	1.0035	0.92	0.92	1.0070	1.82	1.83
1.0001	0.03	0.03	1.0036	0.94	0.94	1.0071	1.84	1.85
1.0002	0.05	0.05	1.0037	0.97	0.97	1.0072	1.87	1.88
1.0003	0.08	0.08	1.0038	1.00	1.00	1.0073	1.90	1.91
1.0004	0.10	0.10	1.0039	1.02	1.02	1.0074	1.92	1.93
1.0005	0.13	0.13	1.0040	1.05	1.05	1.0075	1.95	1.96
1.0006	0.16	0.16	1.0041	1.08	1.08	1.0076	1.97	1.98
1.0007	0.18	0.18	1.0042	1.10	1.10	1.0077	2.00	2.02
1.0008	0.21	0.21	1.0043	1.13	1.13	1.0078	2.02	2.04
1.0009	0.24	0.24	1.0044	1.15	1.16	1.0079	2.05	2.07
1.0010	0.26	0.26	1.0045	1.18	1.19	1.0080	2.07	2.09
1.0011	0.29	0.29	1.0046	1.21	1.22	1.0081	2.10	2.12
1.0012	0.31	0.31	1.0047	1.23	1.24	1.0082	2.12	2.14
1.0013	0.34	0.34	1.0048	1.26	1.27	1.0083	2.15	2.17
1.0014	0.37	0.37	1.0049	1.29	1.30	1.0084	2.17	2.19
1.0015	0.39	0.39	1.0050	1.31	1.32	1.0085	2.20	2.22
1.0016	0.42	0.42	1.0051	1.34	1.35	1.0086	2.23	2.25
1.0017	0.45	0.45	1.0052	1.36	1.37	1.0087	2.25	2.27
1.0018	0.47	0.47	1.0053	1.39	1.40	1.0088	2.28	2.30
1.0019	0.50	0.50	1.0054	1.41	1.42	1.0089	2.30	2.32
1.0020	0.52	0.52	1.0055	1.44	1.45	1.0090	2.33	2.35
1.0021	0.55	0.55	1.0056	1.46	1.47	1.0091	2.35	2.37
1.0022	0.58	0.58	1.0057	1.49	1.50	1.0092	2.38	2.40
1.0023	0.60	0.60	1.0058	1.51	1.52	1.0093	2.41	2.43
1.0024	0.63	0.63	1.0059	1.54	1.55	1.0094	2.43	2.45
1.0025	0.66	0.66	1.0060	1.56	1.57	1.0095	2.46	2.48
1.0026	0.68	0.68	1.0061	1.59	1.60	1.0096	2.48	2.50
1.0027	0.71	0.71	1.0062	1.62	1.63	1.0097	2.51	2.53
1.0028	0.73	0.73	1.0063	1.64	1.65	1.0098	2.53	2.55
1.0029	0.76	0.76	1.0064	1.67	1.68	1.0099	2.56	2.59
1.0030	0.79	0.79	1.0065	1.69	1.70	1.0100	2.58	2.61
1.0031	0.81	0.81	1.0066	1.72	1.73	1.0101	2.61	2.64
1.0032	0.84	0.84	1.0067	1.74	1.75	1.0102	2.64	2.67
1.0033	0.87	0.87	1.0068	1.77	1.78	1.0103	2.66	2.69
1.0034	0.89	0.89	1.0069	1.79	1.80	1.0104	2.69	2.72

⁸⁵ Calculated from results obtained by drying below 76° C.

EXTRACT IN BEER WORT.—*Continued.*

Specific gravity at 15°C.	Extract.		Specific gravity at 15°C.	Extract.		Specific gravity at 15°C.	Extract.	
	Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by weight.	Grams per 100 cubic centimeters.
I.0105	2.71	2.74	I.0145	3.74	3.79	I.0185	4.79	4.88
I.0106	2.74	2.77	I.0146	3.77	3.83	I.0186	4.82	4.91
I.0107	2.76	2.79	I.0147	3.79	3.85	I.0187	4.85	4.94
I.0108	2.79	2.82	I.0148	3.82	3.88	I.0188	4.88	4.97
I.0109	2.82	2.85	I.0149	3.85	3.91	I.0189	4.90	4.99
I.0110	2.84	2.87	I.0150	3.87	3.93	I.0190	4.93	5.02
I.0111	2.87	2.90	I.0151	3.90	3.96	I.0191	4.96	5.05
I.0112	2.89	2.92	I.0152	3.92	3.98	I.0192	4.98	5.08
I.0113	2.92	2.95	I.0153	3.95	4.01	I.0193	5.01	5.11
I.0114	2.94	2.97	I.0154	3.97	4.03	I.0194	5.04	5.14
I.0115	2.97	3.00	I.0155	4.00	4.06	I.0195	5.06	5.16
I.0116	2.99	3.02	I.0156	4.03	4.09	I.0196	5.09	5.19
I.0117	3.02	3.06	I.0157	4.05	4.11	I.0197	5.12	5.22
I.0118	3.05	3.09	I.0158	4.08	4.14	I.0198	5.15	5.25
I.0119	3.07	3.11	I.0159	4.10	4.17	I.0199	5.17	5.27
I.0120	3.10	3.14	I.0160	4.13	4.20	I.0200	5.20	5.30
I.0121	3.12	3.16	I.0161	4.16	4.23	I.0201	5.23	5.34
I.0122	3.15	3.19	I.0162	4.18	4.25	I.0202	5.25	5.36
I.0123	3.17	3.21	I.0163	4.21	4.28	I.0203	5.28	5.39
I.0124	3.20	3.24	I.0164	4.23	4.30	I.0204	5.30	5.41
I.0125	3.23	3.27	I.0165	4.26	4.33	I.0205	5.33	5.44
I.0126	3.25	3.29	I.0166	4.28	4.35	I.0206	5.35	5.46
I.0127	3.28	3.32	I.0167	4.31	4.38	I.0207	5.38	5.49
I.0128	3.30	3.34	I.0168	4.34	4.41	I.0208	5.40	5.51
I.0129	3.33	3.37	I.0169	4.36	4.43	I.0209	5.43	5.54
I.0130	3.35	3.39	I.0170	4.39	4.46	I.0210	5.45	5.56
I.0131	3.38	3.42	I.0171	4.42	4.50	I.0211	5.48	5.60
I.0132	3.41	3.46	I.0172	4.44	4.52	I.0212	5.50	5.62
I.0133	3.43	3.48	I.0173	4.47	4.55	I.0213	5.53	5.65
I.0134	3.46	3.51	I.0174	4.50	4.58	I.0214	5.55	5.67
I.0135	3.48	3.53	I.0175	4.53	4.61	I.0215	5.57	5.69
I.0136	3.51	3.56	I.0176	4.55	4.63	I.0216	5.60	5.72
I.0137	3.54	3.59	I.0177	4.58	4.66	I.0217	5.62	5.74
I.0138	3.56	3.61	I.0178	4.61	4.69	I.0218	5.65	5.77
I.0139	3.59	3.64	I.0179	4.63	4.71	I.0219	5.67	5.79
I.0140	3.61	3.66	I.0180	4.66	4.74	I.0220	5.70	5.83
I.0141	3.64	3.69	I.0181	4.69	4.77	I.0221	5.72	5.85
I.0142	3.66	3.71	I.0182	4.71	4.80	I.0222	5.75	5.88
I.0143	3.69	3.74	I.0183	4.74	4.83	I.0223	5.77	5.90
I.0144	3.72	3.77	I.0184	4.77	4.86	I.0224	5.80	5.93

EXTRACT IN BEER WORT.—*Continued.*

Specific gravity at 15°C.	Extract.		Specific gravity at 15°C.	Extract.		Specific gravity at 15°C.	Extract.	
	Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by weight.	Grams per 100 cubic centimeters.
1.0225	5.82	5.95	1.0265	6.85	7.03	1.0305	7.82	8.06
1.0226	5.84	5.97	1.0266	6.88	7.06	1.0306	7.84	8.08
1.0227	5.87	6.00	1.0267	6.91	7.09	1.0307	7.86	8.10
1.0228	5.89	6.02	1.0268	6.93	7.12	1.0308	7.89	8.13
1.0229	5.92	6.06	1.0269	6.96	7.15	1.0309	7.91	8.15
1.0230	5.94	6.08	1.0270	6.99	7.18	1.0310	7.93	8.18
1.0231	5.97	6.11	1.0271	7.01	7.20	1.0311	7.95	8.20
1.0232	5.99	6.13	1.0272	7.04	7.23	1.0312	7.98	8.23
1.0233	6.02	6.16	1.0273	7.07	7.26	1.0313	8.00	8.25
1.0234	6.04	6.18	1.0274	7.10	7.29	1.0314	8.02	8.27
1.0235	6.07	6.21	1.0275	7.12	7.32	1.0315	8.04	8.29
1.0236	6.09	6.23	1.0276	7.15	7.35	1.0316	8.07	8.33
1.0237	6.11	6.25	1.0277	7.18	7.38	1.0317	8.09	8.35
1.0238	6.14	6.29	1.0278	7.21	7.41	1.0318	8.11	8.37
1.0239	6.16	6.31	1.0279	7.23	7.43	1.0319	8.13	8.39
1.0240	6.19	6.34	1.0280	7.26	7.46	1.0320	8.16	8.42
1.0241	6.21	6.36	1.0281	7.28	7.48	1.0321	8.18	8.44
1.0242	6.24	6.39	1.0282	7.30	7.51	1.0322	8.20	8.46
1.0243	6.26	6.41	1.0283	7.33	7.54	1.0323	8.22	8.49
1.0244	6.29	6.44	1.0284	7.35	7.56	1.0324	8.25	8.52
1.0245	6.31	6.46	1.0285	7.37	7.58	1.0325	8.27	8.54
1.0246	6.34	6.50	1.0286	7.39	7.60	1.0326	8.29	8.56
1.0247	6.36	6.52	1.0287	7.42	7.63	1.0327	8.32	8.59
1.0248	6.39	6.55	1.0288	7.44	7.65	1.0328	8.34	8.61
1.0249	6.41	6.57	1.0289	7.46	7.68	1.0329	8.37	8.65
1.0250	6.44	6.60	1.0290	7.48	7.70	1.0330	8.40	8.68
1.0251	6.47	6.63	1.0291	7.51	7.73	1.0331	8.43	8.71
1.0252	6.50	6.66	1.0292	7.53	7.75	1.0332	8.45	8.73
1.0253	6.52	6.68	1.0293	7.55	7.77	1.0333	8.48	8.76
1.0254	6.55	6.72	1.0294	7.57	7.79	1.0334	8.51	8.79
1.0255	6.58	6.75	1.0295	7.60	7.82	1.0335	8.53	8.82
1.0256	6.61	6.78	1.0296	7.62	7.85	1.0336	8.56	8.85
1.0257	6.63	6.80	1.0297	7.64	7.87	1.0337	8.59	8.88
1.0258	6.66	6.83	1.0298	7.66	7.89	1.0338	8.61	8.90
1.0259	6.69	6.86	1.0299	7.69	7.92	1.0339	8.64	8.93
1.0260	6.71	6.88	1.0300	7.71	7.94	1.0340	8.67	8.96
1.0261	6.74	6.92	1.0301	7.73	7.96	1.0341	8.70	9.00
1.0262	6.77	6.95	1.0302	7.75	7.98	1.0342	8.72	9.02
1.0263	6.80	6.98	1.0303	7.77	8.01	1.0343	8.75	9.05
1.0264	6.82	7.00	1.0304	7.80	8.04	1.0344	8.78	9.08

EXTRACT IN BEER WORT.—*Continued.*

Specific gravity at 15°C.	Extract.		Specific gravity at 15°C.	Extract.		Specific gravity at 15°C.	Extract.	
	Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by weight.	Grams per 100 cubic centimeters.
1.0345	8.80	9.10	1.0385	9.81	10.19	1.0425	10.77	11.23
1.0346	8.83	9.14	1.0386	9.83	10.21	1.0426	10.80	11.26
1.0347	8.86	9.17	1.0387	9.85	10.23	1.0427	10.82	11.28
1.0348	8.88	9.19	1.0388	9.88	10.26	1.0428	10.85	11.31
1.0349	8.91	9.22	1.0389	9.90	10.29	1.0429	10.88	11.35
1.0350	8.94	9.25	1.0390	9.92	10.31	1.0430	10.90	11.37
1.0351	8.97	9.28	1.0391	9.95	10.34	1.0431	10.93	11.40
1.0352	8.99	9.31	1.0392	9.97	10.36	1.0432	10.95	11.42
1.0353	9.02	9.34	1.0393	9.99	10.38	1.0433	10.98	11.46
1.0354	9.05	9.37	1.0394	10.02	10.41	1.0434	11.00	11.48
1.0355	9.07	9.39	1.0395	10.04	10.44	1.0435	11.03	11.51
1.0356	9.10	9.42	1.0396	10.06	10.46	1.0436	11.05	11.53
1.0357	9.13	9.46	1.0397	10.09	10.49	1.0437	11.08	11.56
1.0358	9.15	9.48	1.0398	10.11	10.51	1.0438	11.10	11.59
1.0359	9.18	9.51	1.0399	10.13	10.53	1.0439	11.13	11.62
1.0360	9.21	9.54	1.0400	10.16	10.57	1.0440	11.15	11.64
1.0361	9.24	9.57	1.0401	10.18	10.59	1.0441	11.18	11.67
1.0362	9.26	9.60	1.0402	10.20	10.61	1.0442	11.20	11.70
1.0363	9.29	9.63	1.0403	10.23	10.64	1.0443	11.23	11.73
1.0364	9.31	9.65	1.0404	10.25	10.66	1.0444	11.25	11.75
1.0365	9.34	9.68	1.0405	10.27	10.69	1.0445	11.28	11.78
1.0366	9.36	9.70	1.0406	10.30	10.72	1.0446	11.30	11.80
1.0367	9.38	9.72	1.0407	10.32	10.74	1.0447	11.33	11.84
1.0368	9.41	9.76	1.0408	10.35	10.77	1.0448	11.35	11.86
1.0369	9.43	9.78	1.0409	10.37	10.79	1.0449	11.38	11.89
1.0370	9.45	9.80	1.0410	10.40	10.83	1.0450	11.40	11.91
1.0371	9.48	9.83	1.0411	10.42	10.85	1.0451	11.43	11.95
1.0372	9.50	9.85	1.0412	10.45	10.88	1.0452	11.45	11.97
1.0373	9.52	9.88	1.0413	10.47	10.90	1.0453	11.48	12.00
1.0374	9.55	9.91	1.0414	10.50	10.93	1.0454	11.50	12.02
1.0375	9.57	9.93	1.0415	10.52	10.96	1.0455	11.53	12.05
1.0376	9.59	9.95	1.0416	10.55	10.99	1.0456	11.55	12.08
1.0377	9.62	9.98	1.0417	10.57	11.01	1.0457	11.57	12.10
1.0378	9.64	10.00	1.0418	10.60	11.04	1.0458	11.60	12.13
1.0379	9.66	10.03	1.0419	10.62	11.06	1.0459	11.62	12.15
1.0380	9.69	10.06	1.0420	10.65	11.10	1.0460	11.65	12.19
1.0381	9.71	10.08	1.0421	10.67	11.12	1.0461	11.67	12.21
1.0382	9.73	10.10	1.0422	10.70	11.15	1.0462	11.70	12.24
1.0383	9.76	10.13	1.0423	10.72	11.17	1.0463	11.72	12.26
1.0384	9.78	10.16	1.0424	10.75	11.21	1.0464	11.75	12.30

EXTRACT IN BEER WORT.—Continued.

Specific gravity at 15°C.	Extract.		Specific gravity at 15°C.	Extract.		Specific gravity at 15°C.	Extract.	
	Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by weight.	Grams per 100 cubic centimeters.
1.0465	11.77	12.32	1.0505	12.75	13.39	1.0545	13.73	14.48
1.0466	11.79	12.34	1.0506	12.77	13.42	1.0546	13.76	14.51
1.0467	11.82	12.37	1.0507	12.80	13.45	1.0547	13.78	14.53
1.0468	11.84	12.39	1.0508	12.82	13.47	1.0548	13.81	14.57
1.0469	11.87	12.43	1.0509	12.85	13.50	1.0549	13.83	14.59
1.0470	11.89	12.45	1.0510	12.87	13.53	1.0550	13.86	14.62
1.0471	11.92	12.48	1.0511	12.90	13.56	1.0551	13.88	14.64
1.0472	11.94	12.50	1.0512	12.92	13.58	1.0552	13.91	14.68
1.0473	11.97	12.54	1.0513	12.94	13.60	1.0553	13.93	14.70
1.0474	11.99	12.56	1.0514	12.97	13.64	1.0554	13.96	14.73
1.0475	12.01	12.58	1.0515	12.99	13.66	1.0555	13.98	14.76
1.0476	12.04	12.61	1.0516	13.02	13.69	1.0556	14.01	14.79
1.0477	12.06	12.64	1.0517	13.04	13.71	1.0557	14.03	14.81
1.0478	12.09	12.67	1.0518	13.07	13.75	1.0558	14.06	14.84
1.0479	12.11	12.69	1.0519	13.09	13.77	1.0559	14.08	14.87
1.0480	12.14	12.72	1.0520	13.12	13.80	1.0560	14.11	14.90
1.0481	12.16	12.74	1.0521	13.14	13.82	1.0561	14.13	14.92
1.0482	12.19	12.78	1.0522	13.16	13.85	1.0562	14.16	14.96
1.0483	12.21	12.80	1.0523	13.19	13.88	1.0563	14.18	14.98
1.0484	12.23	12.82	1.0524	13.21	13.90	1.0564	14.21	15.01
1.0485	12.26	12.85	1.0525	13.24	13.94	1.0565	14.23	15.03
1.0486	12.28	12.88	1.0526	13.26	13.96	1.0566	14.26	15.07
1.0487	12.31	12.91	1.0527	13.29	13.99	1.0567	14.28	15.09
1.0488	12.33	12.93	1.0528	13.31	14.01	1.0568	14.31	15.12
1.0489	12.36	12.96	1.0529	13.34	14.05	1.0569	14.33	15.15
1.0490	12.38	12.99	1.0530	13.36	14.07	1.0570	14.36	15.18
1.0491	12.41	13.02	1.0531	13.38	14.09	1.0571	14.38	15.20
1.0492	12.43	13.04	1.0532	13.41	14.12	1.0572	14.41	15.23
1.0493	12.45	13.06	1.0533	13.43	14.15	1.0573	14.44	15.27
1.0494	12.48	13.10	1.0534	13.46	14.18	1.0574	14.46	15.29
1.0495	12.50	13.12	1.0535	13.48	14.20	1.0575	14.49	15.32
1.0496	12.53	13.15	1.0536	13.51	14.23	1.0576	14.52	15.36
1.0497	12.55	13.17	1.0537	13.53	14.26	1.0577	14.54	15.38
1.0498	12.58	13.21	1.0538	13.56	14.29	1.0578	14.57	15.41
1.0499	12.60	13.23	1.0539	13.58	14.31	1.0579	14.59	15.43
1.0500	12.63	13.26	1.0540	13.61	14.34	1.0580	14.62	15.47
1.0501	12.65	13.28	1.0541	13.63	14.37	1.0581	14.65	15.50
1.0502	12.67	13.31	1.0542	13.66	14.40	1.0582	14.67	15.52
1.0503	12.70	13.34	1.0543	13.68	14.42	1.0583	14.70	15.56
1.0504	12.72	13.36	1.0544	13.71	14.46	1.0584	14.73	15.59

EXTRACT IN BEER WORT.—*Continued.*

Specific gravity at 15°C.	Extract.		Specific gravity at 15°C.	Extract.		Specific gravity at 15°C.	Extract.	
	Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by weight.	Grams per 100 cubic centimeters.
1.0585	14.75	15.61	1.0625	15.69	16.66	1.0665	16.62	17.73
1.0586	14.78	15.65	1.0626	15.72	16.70	1.0666	16.64	17.75
1.0587	14.81	15.68	1.0627	15.74	16.73	1.0667	16.67	17.78
1.0588	14.83	15.70	1.0628	15.76	16.75	1.0668	16.69	17.80
1.0589	14.86	15.74	1.0629	15.78	16.77	1.0669	16.72	17.84
1.0590	14.89	15.77	1.0630	15.80	16.80	1.0670	16.74	17.86
1.0591	14.91	15.79	1.0631	15.83	16.83	1.0671	16.76	17.88
1.0592	14.94	15.82	1.0632	15.85	16.85	1.0672	16.79	17.92
1.0593	14.96	15.85	1.0633	15.87	16.87	1.0673	16.81	17.94
1.0594	14.99	15.88	1.0634	15.89	16.90	1.0674	16.84	17.98
1.0595	15.02	15.91	1.0635	15.92	16.93	1.0675	16.86	18.00
1.0596	15.04	15.94	1.0636	15.94	16.95	1.0676	16.89	18.03
1.0597	15.07	15.97	1.0637	15.96	16.98	1.0677	16.91	18.05
1.0598	15.09	15.99	1.0638	15.98	17.00	1.0678	16.94	18.09
1.0599	15.11	16.02	1.0639	16.01	17.03	1.0679	16.96	18.11
1.0600	15.14	16.05	1.0640	16.03	17.06	1.0680	16.99	18.15
1.0601	15.16	16.07	1.0641	16.06	17.08	1.0681	17.01	18.17
1.0602	15.18	16.09	1.0642	16.07	17.10	1.0682	17.03	18.19
1.0603	15.20	16.12	1.0643	16.09	17.12	1.0683	17.06	18.23
1.0604	15.23	16.15	1.0644	16.12	17.16	1.0684	17.08	18.25
1.0605	15.25	16.17	1.0645	16.14	17.18	1.0685	17.11	18.28
1.0606	15.27	16.20	1.0646	16.16	17.20	1.0686	17.13	18.31
1.0607	15.29	16.22	1.0647	16.18	17.23	1.0687	17.16	18.34
1.0608	15.31	16.24	1.0648	16.21	17.26	1.0688	17.18	18.36
1.0609	15.34	16.27	1.0649	16.23	17.28	1.0689	17.21	18.40
1.0610	15.36	16.30	1.0650	16.25	17.31	1.0690	17.23	18.42
1.0611	15.38	16.32	1.0651	16.27	17.33	1.0691	17.25	18.44
1.0612	15.40	16.34	1.0652	16.30	17.36	1.0692	17.28	18.48
1.0613	15.43	16.38	1.0653	16.32	17.39	1.0693	17.30	18.50
1.0614	15.45	16.40	1.0654	16.35	17.42	1.0694	17.33	18.53
1.0615	15.47	16.42	1.0655	16.37	17.44	1.0695	17.35	18.56
1.0616	15.49	16.44	1.0656	16.40	17.48	1.0696	17.38	18.59
1.0617	15.52	16.48	1.0657	16.42	17.50	1.0697	17.40	18.61
1.0618	15.54	16.50	1.0658	16.45	17.53	1.0698	17.43	18.65
1.0619	15.56	16.52	1.0659	16.47	17.56	1.0699	17.45	18.67
1.0620	15.58	16.55	1.0660	16.50	17.59	1.0700	17.48	18.70
1.0621	15.60	16.57	1.0661	16.52	17.61	1.0701	17.50	18.73
1.0622	15.63	16.60	1.0662	16.54	17.63	1.0702	17.52	18.75
1.0623	15.65	16.62	1.0663	16.57	17.67	1.0703	17.54	18.77
1.0624	15.67	16.64	1.0664	16.59	17.69	1.0704	17.57	18.81

EXTRACT IN BEER WORT.—*Continued.*

Specific gravity at 15°C.	Extract.		Specific gravity at 15°C.	Extract.		Specific gravity at 15°C.	Extract.	
	Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by weight.	Grams per 100 cubic centimeters.
1.0705	17.59	18.83	1.0745	18.49	19.87	1.0785	19.33	20.85
1.0706	17.61	18.85	1.0746	18.51	19.89	1.0786	19.36	20.88
1.0707	17.63	18.88	1.0747	18.53	19.91	1.0787	19.38	20.90
1.0708	17.66	18.91	1.0748	18.55	19.94	1.0788	19.40	20.93
1.0709	17.68	18.93	1.0749	18.57	19.96	1.0789	19.42	20.95
1.0710	17.70	18.96	1.0750	18.59	19.98	1.0790	19.41	20.98
1.0711	17.72	18.98	1.0751	18.62	20.02	1.0791	19.46	21.00
1.0712	17.75	19.01	1.0752	18.64	20.04	1.0792	19.49	21.03
1.0713	17.77	19.04	1.0753	18.66	20.07	1.0793	19.51	21.06
1.0714	17.79	19.06	1.0754	18.68	20.09	1.0794	19.53	21.08
1.0715	17.81	19.08	1.0755	18.70	20.11	1.0795	19.56	21.11
1.0716	17.84	19.12	1.0756	18.72	20.14	1.0796	19.58	21.14
1.0717	17.86	19.14	1.0757	18.74	20.16	1.0797	19.60	21.16
1.0718	17.88	19.15	1.0758	18.76	20.18	1.0798	19.63	21.20
1.0719	17.90	19.19	1.0759	18.78	20.21	1.0799	19.65	21.22
1.0720	17.93	19.22	1.0760	18.81	20.24	1.0800	19.67	21.24
1.0721	17.95	19.24	1.0761	18.83	20.26	1.0801	19.70	21.28
1.0722	17.97	19.27	1.0762	18.85	20.29	1.0802	19.72	21.30
1.0723	17.99	19.29	1.0763	18.87	20.31	1.0803	19.74	21.33
1.0724	18.02	19.32	1.0764	18.89	20.33	1.0804	19.77	21.36
1.0725	18.04	19.35	1.0765	18.91	20.36	1.0805	19.79	21.38
1.0726	18.06	19.37	1.0766	18.93	20.38	1.0806	19.81	21.41
1.0727	18.08	19.39	1.0767	18.95	20.40	1.0807	19.84	21.43
1.0728	18.11	19.43	1.0768	18.97	20.43	1.0808	19.86	21.46
1.0729	18.13	19.45	1.0769	19.00	20.46	1.0809	19.88	21.49
1.0730	18.15	19.47	1.0770	19.02	20.48	1.0810	19.91	21.52
1.0731	18.17	19.50	1.0771	19.04	20.51	1.0811	19.93	21.55
1.0732	18.20	19.53	1.0772	19.06	20.53	1.0812	19.96	21.58
1.0733	18.22	19.55	1.0773	19.08	20.55	1.0813	19.98	21.60
1.0734	18.24	19.58	1.0774	19.10	20.58	1.0814	20.00	21.63
1.0735	18.26	19.60	1.0775	19.12	20.60	1.0815	20.03	21.66
1.0736	18.29	19.64	1.0776	19.14	20.63	1.0816	20.05	21.69
1.0737	18.31	19.66	1.0777	19.17	20.66	1.0817	20.07	21.71
1.0738	18.33	19.68	1.0778	19.19	20.68	1.0818	20.10	21.74
1.0739	18.35	19.71	1.0779	19.21	20.71	1.0819	20.12	21.77
1.0740	18.38	19.74	1.0780	19.23	20.73	1.0820	20.14	21.79
1.0741	18.40	19.76	1.0781	19.25	20.75	1.0821	20.17	21.83
1.0742	18.42	19.79	1.0782	19.27	20.78	1.0822	20.19	21.85
1.0743	18.44	19.81	1.0783	19.29	20.80	1.0823	20.21	21.87
1.0744	18.47	19.84	1.0784	19.31	20.82	1.0824	20.24	21.91

EXTRACT IN BEER WORT.—*Continued.*

Specific gravity at 15°C.	Extract.		Specific gravity at 15°C.	Extract.		Specific gravity at 15°C.	Extract.	
	Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by weight.	Grams per 100 cubic centimeters.
I.0825	20.26	21.93	I.0865	21.19	23.02	I.0905	22.08	24.08
I.0826	20.28	21.96	I.0866	21.22	23.06	I.0906	22.10	24.11
I.0827	20.31	21.99	I.0867	21.25	23.09	I.0907	22.12	24.13
I.0828	20.33	22.01	I.0868	21.28	23.12	I.0908	22.15	24.16
I.0829	20.35	22.04	I.0869	21.30	23.15	I.0909	22.17	24.18
I.0830	20.37	22.06	I.0870	21.33	23.18	I.0910	22.19	24.21
I.0831	20.39	22.08	I.0871	21.35	23.21	I.0911	22.21	24.24
I.0832	20.41	22.11	I.0872	21.37	23.23	I.0912	22.23	24.26
I.0833	20.43	22.13	I.0873	21.39	23.26	I.0913	22.26	24.29
I.0834	20.46	22.16	I.0874	21.41	23.28	I.0914	22.28	24.31
I.0835	20.48	22.19	I.0875	21.43	23.31	I.0915	22.30	24.34
I.0836	20.50	22.21	I.0876	21.45	23.33	I.0916	22.32	24.37
I.0837	20.52	22.24	I.0877	21.47	23.36	I.0917	22.34	24.39
I.0838	20.54	22.26	I.0878	21.49	23.38	I.0918	22.37	24.42
I.0839	20.56	22.29	I.0879	21.51	23.40	I.0919	22.39	24.44
I.0840	20.59	22.32	I.0880	21.54	23.43	I.0920	22.41	24.47
I.0841	20.62	22.35	I.0881	21.56	23.45	I.0921	22.43	24.49
I.0842	20.64	22.38	I.0882	21.58	23.48	I.0922	22.45	24.51
I.0843	20.66	22.40	I.0883	21.60	23.50	I.0923	22.48	24.54
I.0844	20.68	22.42	I.0884	21.62	23.52	I.0924	22.50	24.56
I.0845	20.70	22.45	I.0885	21.64	23.55	I.0925	22.52	24.60
I.0846	20.73	22.48	I.0886	21.66	23.58	I.0926	22.54	24.62
I.0847	20.75	22.50	I.0887	21.68	23.60	I.0927	22.56	24.64
I.0848	20.77	22.53	I.0888	21.71	23.63	I.0928	22.59	24.67
I.0849	20.79	22.55	I.0889	21.73	23.66	I.0929	22.61	24.70
I.0850	20.81	22.58	I.0890	21.75	23.69	I.0930	22.63	24.73
I.0851	20.83	22.61	I.0891	21.77	23.72	I.0931	22.65	24.76
I.0852	20.86	22.64	I.0892	21.79	23.74	I.0932	22.67	24.78
I.0853	20.88	22.66	I.0893	21.82	23.77	I.0933	22.69	24.81
I.0854	20.90	22.68	I.0894	21.84	23.79	I.0934	22.71	24.83
I.0855	20.93	22.72	I.0895	21.86	23.82	I.0935	22.73	24.86
I.0856	20.95	22.75	I.0896	21.89	23.85	I.0936	22.75	24.89
I.0857	20.98	22.78	I.0897	21.91	23.87	I.0937	22.77	24.91
I.0858	21.01	22.81	I.0898	21.93	23.90	I.0938	22.80	24.93
I.0859	21.04	22.84	I.0899	21.96	23.93	I.0939	22.82	24.96
I.0860	21.06	22.87	I.0900	21.98	23.96	I.0940	22.84	24.99
I.0861	21.09	22.90	I.0901	22.00	23.98	I.0941	22.86	25.01
I.0862	21.11	22.93	I.0902	22.02	24.01	I.0942	22.88	25.03
I.0863	21.13	22.96	I.0903	22.04	24.03	I.0943	22.90	25.06
I.0864	21.16	22.99	I.0904	22.06	24.05	I.0944	22.92	25.08

EXTRACT IN BEER WORT

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EXTRACT IN BEER WORT.—*Continued.*

Specific gravity at 15°C.	Extract.		Specific gravity at 15°C.	Extract.		Specific gravity at 15°C.	Extract.	
	Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by weight.	Grams per 100 cubic centimeters.
1.0945	22.94	25.11	1.0985	23.80	26.14	1.1025	24.64	27.17
1.0946	22.96	25.14	1.0986	23.82	26.17	1.1026	24.66	27.19
1.0947	22.98	25.16	1.0987	23.84	26.19	1.1027	24.68	27.21
1.0948	23.00	25.18	1.0988	23.86	26.22	1.1028	24.70	27.24
1.0949	23.03	25.21	1.0989	23.88	26.24	1.1029	24.72	27.26
1.0950	23.05	25.24	1.0990	23.90	26.27	1.1030	24.74	27.29
1.0951	23.07	25.26	1.0991	23.92	26.30	1.1031	24.76	27.32
1.0952	23.10	25.29	1.0992	23.94	26.32	1.1032	24.78	27.34
1.0953	23.12	25.31	1.0993	23.97	26.35	1.1033	24.81	27.37
1.0954	23.14	25.34	1.0994	23.99	26.37	1.1034	24.83	27.39
1.0955	23.16	25.37	1.0995	24.01	26.40	1.1035	24.85	27.42
1.0956	23.18	25.39	1.0996	24.03	26.42	1.1036	24.87	27.45
1.0957	23.20	25.42	1.0997	24.05	26.44	1.1037	24.89	27.47
1.0958	23.23	25.45	1.0998	24.07	26.47	1.1038	24.92	27.50
1.0959	23.25	25.47	1.0999	24.09	26.49	1.1039	24.94	27.53
1.0960	23.27	25.50	1.1000	24.11	26.52	1.1040	24.96	27.56
1.0961	23.29	25.53	1.1001	24.13	26.55	1.1041	24.98	27.58
1.0961	23.31	25.55	1.1002	24.15	26.57	1.1042	25.00	27.60
1.0963	23.33	25.58	1.1003	24.17	26.60	1.1043	25.03	27.63
1.0964	23.35	25.60	1.1004	24.19	26.62	1.1044	25.05	27.66
1.0965	23.37	25.63	1.1005	24.21	26.65	1.1045	25.07	27.69
1.0966	23.39	25.66	1.1006	24.23	26.68	1.1046	25.09	27.72
1.0967	23.41	25.68	1.1007	24.25	26.70	1.1047	25.11	27.74
1.0968	23.44	25.71	1.1008	24.28	26.73	1.1048	25.14	27.77
1.0969	23.46	25.73	1.1009	24.30	26.75	1.1049	25.16	27.79
1.0970	23.48	25.76	1.1010	24.32	26.78	1.1050	25.18	27.82
1.0971	23.50	25.79	1.1011	24.34	26.81	1.1051	25.20	27.85
1.0972	23.52	25.81	1.1011	24.36	26.83	1.1052	25.22	27.87
1.0973	23.55	25.84	1.1013	24.39	26.89	1.1053	25.24	27.90
1.0974	23.57	25.86	1.1014	24.41	26.88	1.1054	25.27	27.93
1.0975	23.59	25.89	1.1015	24.43	26.91	1.1055	25.29	27.96
1.0976	23.61	25.92	1.1016	24.45	26.93	1.1056	25.31	27.98
1.0977	23.63	25.94	1.1017	24.47	26.95	1.1057	25.33	28.00
1.0978	23.65	25.97	1.1018	24.49	26.98	1.1058	25.35	28.03
1.0979	23.67	25.99	1.1019	24.51	27.00	1.1059	25.38	28.06
1.0980	23.69	26.01	1.1020	24.53	27.03	1.1060	25.40	28.09
1.0981	23.71	26.04	1.1021	24.55	27.06	1.1061	25.42	28.12
1.0982	23.73	26.06	1.1022	24.57	27.08	1.1062	25.44	28.14
1.0983	23.76	26.09	1.1023	24.60	27.11	1.1063	25.46	28.17
1.0984	23.78	26.11	1.1024	24.62	27.14	1.1064	25.48	28.19

EXTRACT IN BEER WORT.—*Continued.*

Specific gravity at 15°C.	Extract.		Specific gravity at 15°C.	Extract.		Specific gravity at 15°C.	Extract.	
	Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by weight.	Grams per 100 cubic centimeters.
I.1065	25.50	28.22	I.1105	26.37	29.29	I.1145	27.19	30.31
I.1066	25.52	28.25	I.1106	26.39	29.32	I.1146	27.21	30.33
I.1067	25.54	28.27	I.1107	26.41	29.34	I.1147	27.23	30.35
I.1068	25.57	28.30	I.1108	26.44	29.37	I.1148	27.25	30.37
I.1069	25.59	28.32	I.1109	26.46	29.39	I.1149	27.27	30.40
I.1070	25.61	28.35	I.1110	26.48	29.42	I.1150	27.29	30.43
I.1071	25.63	28.38	I.1111	26.50	29.44	I.1151	27.31	30.45
I.1072	25.65	28.40	I.1112	26.52	29.46	I.1152	27.33	30.47
I.1073	25.67	28.43	I.1113	26.54	29.49	I.1153	27.35	30.50
I.1074	25.69	28.45	I.1114	26.56	29.51	I.1154	27.37	30.52
I.1075	25.71	28.48	I.1115	26.58	29.54	I.1155	27.38	30.55
I.1076	25.73	28.51	I.1116	26.60	29.57	I.1156	27.40	30.57
I.1077	25.75	28.53	I.1117	26.62	29.59	I.1157	27.42	30.59
I.1078	25.78	28.56	I.1118	26.64	29.61	I.1158	27.44	30.62
I.1079	25.80	28.58	I.1119	26.66	29.64	I.1159	27.46	30.64
I.1080	25.82	28.61	I.1120	26.68	29.67	I.1160	27.48	30.67
I.1081	25.84	28.64	I.1121	26.70	29.69	I.1161	27.50	30.69
I.1082	25.86	28.66	I.1122	26.72	29.71	I.1162	27.52	30.72
I.1083	25.89	28.69	I.1123	26.75	29.74	I.1163	27.54	30.75
I.1084	25.91	28.72	I.1124	26.77	29.77	I.1164	27.56	30.77
I.1085	25.93	28.75	I.1125	26.79	29.80	I.1165	27.58	30.80
I.1086	25.96	28.78	I.1126	26.81	29.83	I.1166	27.60	30.82
I.1087	25.98	28.80	I.1127	26.83	29.85	I.1167	27.62	30.85
I.1088	26.01	28.83	I.1128	26.85	29.88	I.1168	27.64	30.87
I.1089	26.03	28.86	I.1129	26.87	29.90	I.1169	27.66	30.89
I.1090	26.05	28.89	I.1130	26.89	29.93	I.1170	27.68	30.92
I.1091	26.07	28.92	I.1131	26.91	29.95	I.1171	27.70	30.94
I.1092	26.09	28.94	I.1132	26.93	29.97	I.1172	27.72	30.97
I.1093	26.12	28.97	I.1133	26.95	30.00	I.1173	27.74	31.00
I.1094	26.14	29.00	I.1134	26.97	30.02	I.1174	27.76	31.02
I.1095	26.16	29.03	I.1135	26.99	30.06	I.1175	27.78	31.05
I.1096	26.18	29.06	I.1136	27.01	30.08	I.1176	27.80	31.07
I.1097	26.20	29.08	I.1137	27.03	30.10	I.1177	27.82	31.09
I.1098	26.23	29.11	I.1138	27.05	30.13	I.1178	27.84	31.12
I.1099	26.25	29.13	I.1139	27.07	30.15	I.1179	27.86	31.15
I.1100	26.27	29.16	I.1140	27.09	30.18	I.1180	27.88	31.18
I.1101	26.29	29.19	I.1141	27.11	30.20	I.1181	27.90	31.20
I.1102	26.31	29.21	I.1142	27.13	30.22	I.1182	27.92	31.23
I.1103	26.33	29.24	I.1143	27.15	30.25	I.1183	27.94	31.25
I.1104	26.35	29.26	I.1144	27.17	30.27	I.1184	27.96	31.27

EXTRACT IN BEER WORT.—*Continued.*

Specific gravity at 15°C.	Extract.		Specific gravity at 15°C.	Extract.		Specific gravity at 15°C.	Extract.	
	Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by weight.	Grams per 100 cubic centimeters.		Per cent. by weight.	Grams per 100 cubic centimeters.
1.1185	27.98	31.30	1.1210	28.48	31.93	1.1235	28.98	32.56
1.1186	28.00	31.32	1.1211	28.50	31.95	1.1236	29.00	32.58
1.1187	28.02	31.35	1.1212	28.52	31.98	1.1237	29.02	32.60
1.1188	28.04	31.37	1.1213	28.54	32.00	1.1238	29.04	32.63
1.1189	28.07	31.40	1.1214	28.56	32.03	1.1239	29.06	32.65
1.1190	28.09	31.43	1.1215	28.58	32.05	1.1240	29.08	32.68
1.1191	28.11	31.45	1.1216	28.60	32.08	1.1241	29.10	32.71
1.1192	28.13	31.48	1.1217	28.62	32.11	1.1242	29.12	32.73
1.1193	28.15	31.51	1.1218	28.64	32.13	1.1243	29.14	32.76
1.1194	28.17	31.53	1.1219	28.66	32.15	1.1244	29.16	32.78
1.1195	28.19	31.56	1.1220	28.68	32.18	1.1245	29.18	32.81
1.1196	28.21	31.59	1.1221	28.70	32.20	1.1246	29.20	32.83
1.1197	28.23	31.61	1.1222	28.72	32.23	1.1247	29.22	32.86
1.1198	28.25	31.63	1.1223	28.74	32.25	1.1248	29.24	32.89
1.1199	28.27	31.65	1.1224	28.76	32.27	1.1249	29.26	32.91
1.1200	28.28	31.68	1.1225	28.78	32.30	1.1250	29.28	32.94
1.1201	28.30	31.70	1.1226	28.80	32.32	1.1251	29.30	32.96
1.1202	28.32	31.73	1.1227	28.82	32.35	1.1252	29.32	32.99
1.1203	28.34	31.75	1.1228	28.84	32.37	1.1253	29.34	33.02
1.1204	28.36	31.78	1.1229	28.86	32.40	1.1254	29.36	33.04
1.1205	28.38	31.81	1.1230	28.88	32.43	1.1255	29.38	33.07
1.1206	28.40	31.83	1.1231	28.90	32.45	1.1256	29.40	33.09
1.1207	28.42	31.86	1.1232	28.92	32.48	1.1257	29.42	33.12
1.1208	28.44	31.88	1.1233	28.94	32.50	1.1258	29.45	33.14
1.1209	28.46	31.90	1.1234	28.96	32.53	1.1259	29.47	33.17

666. Determination in a Vacuum.—To avoid the changes and decomposition produced by heating, the non volatile bodies may also be determined by drying the sample in a vacuum over sulfuric acid. It has been found that the process may be greatly facilitated by connecting the desiccating apparatus with a vacuum service in which a vacuum corresponding to a mercurial column of 600 millimeters is obtained. The desiccator is provided with a valve whereby a minute current of dry air is allowed to flow through it. This current is not large enough to lessen the vacuum but is sufficient to greatly accelerate the rapidity of

the evaporation. The evaporation is hastened also, in a marked degree, by absorbing the liquid with a piece of filter paper previously dried in a vacuum. When it is desired to examine the residue, however, it must be obtained in a flat dish exposing a large surface to evaporation.

667. Estimation of Water.—It is evident that the percentage of water in a fermented beverage is easily calculated when the percentage of alcohol by weight and that of the dry residue are known. In a given case, if the number of grams of alcohol in 100 of the sample be five and that of fixed solids four and a half, the quantity of water therein is $100 - (5.0 + 4.5) = 90.5$ grams. In this case the volatile essences are counted as water, but these, at most, are so small in quantity as to be practically unweighable. Nevertheless, it must be admitted that direct drying, in many cases, may give erroneous results, especially when the sample contains an abundance of ethers, invert sugar and of glycerol.

668. Total Acidity.—The acidity found in fermented beverages is due both to the natural acids of the materials from which they are made, and to those caused by fermentation. The typical acids also indicate the nature of the original materials, as malic in cider and tartaric in wine. The acids of beers are due almost exclusively to fermentation, and lactic is probably the dominant one. In determining total acidity, it is not always convenient to ascertain beforehand what acid predominates, nor to accurately distribute the acid among its various components. In the analytical work it is advisable, therefore, to estimate the total acid of cider as malic, of wines as tartaric and of beers as lactic. The process of titration is conducted as follows:

Transfer 25 cubic centimeters to a beaker, heat to incipient boiling to expel carbon dioxid, and, in the case of white wine and beer, add about 10 drops of a neutral litmus solution. With red wine add decinormal sodium hydroxid solution until the red color changes to violet. Then add the hydroxid, a few drops at a time, until a drop of the liquid, placed on delicate red litmus paper, shows an alkaline reaction.

One cubic centimeter of decinormal sodium hydroxid solution = 0.0075 gram tartaric, 0.0067 of malic and 0.009 gram of lactic acid.

669. Determination of Volatile Acids.—Fifty cubic centimeters of the sample, to which a little tannin has been added to prevent foaming, are distilled in a current of steam. The flask is heated until the liquid boils, when the lamp under it is turned down and the steam passed through until 200 cubic centimeters have been collected in the receiver. The distillate is titrated with decinormal sodium hydroxid solution and the result expressed as acetic acid, or as lactic, malic or tartaric as desired.

One cubic centimeter of decinormal sodium hydroxid solution = 0.0060 gram acetic acid.

The acidity due to volatile acids may be determined by ascertaining the total acidity as above described, evaporating 100 cubic centimeters to one-third of their volume, restoring the original volume with water and again titrating. The difference between the first and second titrations represents the volatile acidity.

A method of determining volatile acidity in wines, without the application of heat, has been proposed by de la Source.⁸⁶ The sample, five cubic centimeters, freed of carbon dioxid by shaking, is placed in a flat dish about eight centimeters in diameter. In a separate portion of the sample the total acidity is determined in the presence of phenolphthalein by a set solution of barium hydroxid, one cubic centimeter of which is equal to four milligrams of sulfuric acid. The sample in the flat dish is placed in a desiccator, which contains both sulfuric acid and solid potassium hydroxid, and left for two days, by which time it is practically dry. The residue is dissolved in two cubic centimeters of warm water and the dish is kept in the desiccator for an additional two days. By this time the volatile acids, even acetic, will have disappeared and the residual acidity is determined after solution in water.

The method is also applicable when wines have been treated

⁸⁶ The Analyst, 1896, 21 : 158.

with an alkali. In this case two samples of five cubic centimeters each are acidified with two cubic centimeters of a solution of tartaric acid containing 25 grams per liter. This treatment sets free the volatile acids, and their quantity is determined as before.

670. Titration with Phenolphthalein.—The total acidity is also easily determined by titration with a set alkali, using phenolphthalein as indicator. Colored liquors must be treated with animal black before the analysis. The sample is shaken to expel carbon dioxid and five cubic centimeters added to 100 of water containing phenolphthalein. The set alkali (tenth normal sodium hydroxid) is added until the red color is discharged. Even wines having a considerable degree of color may be titrated in this way.⁸⁷ The acidity, expressed as tartaric, may be stated as due to sulfuric by dividing by 1.53.

671. Determination of Tartaric Acid.—The determination of potassium bitartrate is necessary when an estimation of the free tartaric acid is desired. The official method is conducted as follows:⁸⁸

Tartaric Acid and Tartrates.—(a) *Total Tartaric Acid.*—To 100 cubic centimeters of wine add two cubic centimeters of glacial acetic acid, three drops of a 20 per cent. solution of potassium acetate, and 15 grams of powdered potassium chlorid, and stir to hasten solution. Add 15 cubic centimeters of 95 per cent. alcohol and rub the side of the beaker vigorously with a glass rod for about one minute to start crystallization. Let stand at least 15 hours at room temperature; decant the liquid from the separated acid potassium tartrate as rapidly as possible on a gooch prepared with a very thin film of asbestos, transferring no more of the precipitate to the crucible than necessary. Wash the precipitate and filter three times with a small amount of a mixture of 15 grams of potassium chlorid, 20 cubic centi-

⁸⁷ Gautier; *Sophistication et Analyse des Vins*, 1891 : 98.

⁸⁸ Bureau of Chemistry, U. S. Department of Agriculture, Bulletin 107 (revised), 1912 : 86.

Halenke and Moslinger; *Zeitschrift für analytische chemie*, 1895, 34 : 263-293.

meters of 95 per cent. alcohol, and 100 cubic centimeters of water, using not more than 20 cubic centimeters of the wash solution in all. Transfer the asbestos film and precipitate to the beaker in which the precipitation took place, wash out the gooch with hot water, add about 50 cubic centimeters of hot water, heat to boiling, and titrate the hot solution with tenth-normal sodium hydroxid, using delicate litmus tincture or litmus paper as indicator. Increase the number of cubic centimeters of tenth-normal alkali employed by 1.5 cubic centimeters on account of the solubility of the precipitate. One cubic centimeter of tenth-normal alkali so consumed is equivalent to 0.0150 gram of tartaric acid.

(b) *Cream of Tartar*.—Ash 25 cubic centimeters of the vinegar, extract the ash repeatedly with hot water on an ashless filter, dry and ignite the filter with the undissolved residue, cool, weigh, and calculate as insoluble ash. Titrate the aqueous extract with tenth-normal acid, using methyl orange as indicator. Each cubic centimeter of tenth-normal alkali employed is equivalent to 0.0188 gram of potassium bitartrate.

672. Modification of Hartmann and Eoff.—In the official method the tartaric acid in both the free and combined states is precipitated as acid potassium tartrate and ultimately titrated. That the method gives reasonably accurate results in all cases where free tartaric acid is absent has been demonstrated, but it is unreliable where large amounts of free tartaric acid are present in the liquid. This discrepancy is to be attributed to the fact that the principal reaction in the procedure is reversible, potassium chlorid and tartaric acid giving potassium acid tartrate and free hydrochloric acid, an equilibrium taking place between all the members of the equation, and thus withdrawing an appreciable amount of tartaric acid from the precipitation. The amount of tartaric acid lost depends altogether upon the percentage of free tartaric acid present. To offset the decomposing action of the hydrochloric acid, a solution of potassium acetate is added, whereby potassium chlorid and acetic acid are formed. Upon the addition of this salt the usefulness of the method is in the

main dependent, the amount of potassium acetate to be added depending upon the amount of hydrochloric acid liberated in the main reaction. Since, however, the free tartaric acid content of the liquid to be examined is unknown, the amount of potassium acetate to be used would either have to be determined empirically, by using increasing amounts of the salt and accepting that quantity of the salt as correct which admits of the highest percentage of total tartaric acid, or by determining the free tartaric acid approximately and thus fixing the amount of potassium acetate to be added. This method, however, would be too tedious of manipulation to be of value to the wine chemist. It is doubtful if this procedure would make the method trustworthy, because, even in such a case, the disturbing influence of the free hydrochloric acid would not be entirely eliminated.

The temperature at which the mixture is held for 15 hours is of the utmost consequence. Duplicate determinations in which room temperature and a temperature of 5° were maintained, showed about a 10 per cent. increase in tartaric acid in favor of the lower temperature.

From what has been said it is evident that the official method in wines is hardly a desirable method and still less so when it is applied to grape juices high in free tartaric acid.

Realizing the drawbacks of this method and the utter uselessness of the same in grape juice work, the authors endeavored to modify the procedure by eliminating the formation of the objectionable hydrochloric acid. To this end the following method was used in the enological laboratory, Bureau of Chemistry.

To 100 cubic centimeters of wine or grape juice neutralized with normal sodium hydroxid, add the equivalent of tartaric acid corresponding to the soda necessary in the titration, and record this amount. This converts all the acids into acid salts. Add 15 grams of potassium chlorid, two of glacial acetic acid and 15 cubic centimeters alcohol for wines (20 cubic centimeters alcohol for grape juices). After stirring vigorously for a short time, let stand at a temperature between 15° and 20° for 15 hours.

Filter, and wash with alcohol-potassium chlorid. The correction of 1.5 cubic centimeter N/10 soda is maintained as in the official method. The purity of the tartaric acid added should be carefully ascertained in order to make the proper subtractions.

A very decided advantage of the modified method is shown in cases where a very low tartaric acid content is found. In such cases the acid potassium tartrate forms slowly and may even refuse to precipitate altogether. The addition of the tartaric acid required in the modified method hastens the precipitation greatly, and admits of a more complete reaction. It is hardly necessary to say that the method will not determine less than 0.02 grams of tartaric acid per 100 cubic centimeters because of the solubility of the potassium acid tartrate in the concentration maintained. In such cases several hundred cubic centimeters of the wine should be evaporated to 100 cubic centimeters and the method applied on the concentrated solution.

Instead of adding tartaric acid the determination may be made by half neutralizing the acids present with normal sodium hydrate and then proceeding with the potassium chlorid, acetic acid and alcohol additions as described.

From all these considerations the following simplified procedure for the determination of tartaric acid in wines and grape juices is proposed:

Determine the acidity of the wine or juice by titrating with tenth normal sodium hydrate, using litmus solution as an outside indicator. If 20 cubic centimeters of the material under examination require less than 25 cubic centimeters of the tenth normal sodium hydrate, determine the tartaric acid by adding tartaric acid as described in the first method. If the 20 cubic centimeters require more than 25 cubic centimeters tenth normal sodium hydrate, half neutralize and proceed without addition of tartaric acid.

Since pectin bodies and gums which are contained in hot pressed grape juices in considerable amounts make the washing of the potassium acid tartrate crystals very difficult, the tartaric acid content often is exaggerated. It is necessary, therefore, to

remove the pectin bodies before determining the tartaric acid in such juices. This may be accomplished by transferring 100 cubic centimeters of the juice to a liter evaporating dish and heating on a boiling water-bath for half an hour. The water evaporated is replaced and 50 cubic centimeters water added, and the whole boiled for a few minutes. Transfer to a 200 cubic centimeter flask, and after cooling, make to volume and filter. Determine the tartaric acid in 100 cubic centimeters of this solution by neutralizing with normal sodium hydrate and adding tartaric acid, as described in the first method. (672.)

673. Polarizing Bodies in Fermented Beverages.—The study of the nature of the carbohydrates, which constitute an important part of the solid matters dissolved in fermented beverages, is of the greatest importance. These bodies consist of invert sugar, sucrose,⁸⁹ tartaric acid and the unfermented hydrolytic products derived from starch. The natural "grape sugar" which is found in wines, is composed almost entirely of invert sugar. Sucrose is also a very important added constituent of sweet wines. The hydrolytic products of starch are found in beers, either as a residue from the fermentation of malt or from the rice, hominy grits, etc., added in brewing. The character and quantities of these residues can be determined by the methods already given in the parts of this volume relating to sugars and starches.

674. Determination of Glycerol.—*Provisional Official Method.*⁹⁰
—(a) *In Dry Wines.*—Evaporate 100 cubic centimeters of wine in a porcelain dish on the water bath to a volume of about 10 cubic centimeters and treat the residue with about five grams of fine sand and with from three to four cubic centimeters of milk of lime (containing about 15 per cent. of calcium oxid) for each gram of extract present, and evaporate almost to dryness. Treat the moist residue with 50 cubic centimeters of 90 per cent. alcohol by volume, remove the substance adhering to the sides of the dish with a spatula, and rub the whole mass to a

⁸⁹ Alwood; Enological studies, Bureau of Chemistry, U. S. Department of Agriculture, Bulletin 140, 1911: 8-23.

⁹⁰ Bureau of Chemistry, U. S. Department of Agriculture, Bulletin 107 (revised), 1912: 83-84.

paste. Heat the mixture on the water bath, with constant stirring, to incipient boiling and decant the liquid through a filter into a small flask. Wash the residue repeatedly by decantation with 10 cubic centimeter portions of hot 90 per cent. alcohol until the filtrate amounts to about 150 cubic centimeters. Evaporate the filtrate to a sirupy consistency in a porcelain dish, on a hot, but not boiling water bath; transfer the residue to a small glass-stoppered graduated cylinder with 20 cubic centimeters of absolute alcohol, and add three portions of 10 cubic centimeters each of absolute ether, thoroughly shaking after each addition. Let stand until clear, then pour off through a filter, and wash the cylinder and filter with a mixture of one part of absolute alcohol to one and one-half parts of absolute ether, pouring the wash liquor also through the filter. Evaporate the filtrate to a sirupy consistency, dry for one hour at the temperature of boiling water, weigh, ignite, and weigh again. The loss on ignition gives the weight of glycerol.

(b) *In Sweet Wines*.—With wines whose extract exceeds five grams per 100 cubic centimeters, heat to boiling in a flask the portion to be used in the determination of glycerol, and treat with successive small portions of milk of lime until it becomes first darker and then lighter in color. When cool add 200 cubic centimeters of 95 per cent. alcohol, allow the precipitate to subside, filter, and wash with 95 per cent. alcohol. With the filtrates thus obtained proceed as directed under (a).

In Beer.—Proceed as directed above under "Wine." The milk of lime is added during evaporation after the carbon dioxide has been expelled. It is advisable that the filtrate after being evaporated to a sirupy consistency be treated again with five cubic centimeters of absolute alcohol and two portions of 7.5 cubic centimeters each of absolute ether. If clear, continue as directed. If not clear, it is necessary to repeat the treatment with lime.

675. Determination of Ash.—The residue from the direct extract determination is incinerated at as low a heat as possible. Repeated moistening, drying and heating to low redness is advis-

able to get rid of all organic substances. When a quantitative analysis of the ash is desired, large quantities of the sample are evaporated to dryness and the residue incinerated with the usual precautions.

676. Determination of Potash.—(a) *Kayser's Method*.—Dissolve seven-tenths gram pure sodium hydroxid and two grams of tartaric acid in 100 cubic centimeters of wine, add 150 cubic centimeters of 92 to 94 per cent. alcohol and allow the liquid to stand 24 hours. The precipitated potassium bitartrate is collected on a small filter and washed with 50 per cent. alcohol until the filtrate amounts to 260 cubic centimeters. The precipitate and filter are transferred to the beaker in which the precipitation was made, the precipitate dissolved in hot water, the volume made up to 200 cubic centimeters and 50 cubic centimeters thereof titrated with decinormal sodium hydroxid solution, adding 0.004 gram to the final result, representing the potash which remains in solution as bitartrate.

(b) *Platinum Chlorid Method*.—Evaporate 100 cubic centimeters of the wine to dryness, incinerate the residue and determine the potash as in ash analysis.⁹¹

677. Detection of Preservatives.—(1. *Salicylic Acid*⁹²).—A trace of salicylic acid is said to occur naturally in some fruits, and not more than 50 grams should be used for its qualitative detection in the examination of foods. A reaction obtained with this amount is due to added salicylic acid. The method described below is intended for the quantitative determination of salicylic acid. If only a qualitative determination be desired, many of the details may be omitted.

If the material be a solid or semi-solid, rub the sample in a mortar with water made slightly alkaline and strain through a cotton bag or separate by means of a centrifuge. If preferred, rub from 200 to 300 grams with about 400 cubic centimeters of water and use aliquots of the filtrate for the determination of preservatives.

In quantitative work place the mashed mass in a graduated

⁹¹ This work, 2d ed., 1906, 2 : 615.

⁹² Bureau of Chemistry, Bulletin 107 (revised), 179 et. seq.

flask, make up to a definite volume with water, and shake from time to time until solution is complete. Then strain as directed above and use an aliquot of the filtrate for extraction.

Extract in a separatory funnel 100 cubic centimeters of the sample or of the aqueous solution prepared from the sample as described above with a sufficient amount of sulfuric ether to prevent emulsion after the addition of two or three cubic centimeters of dilute (1-3) sulfuric acid. If the nature of the substance is such that extraction with organic solvents is not practicable, as in the case of the presence of a large amount of fat, the salicylic acid may first be separated by distillation. In such cases acidify the comminuted material with phosphoric acid and transfer to a distilling flask with a very short neck and wide mouth. An Erlenmeyer flask with inside diameter of mouth of one and one-fourth inches is a good shape. The tube connecting the flask with condenser should be very short, with an inside diameter of not less than three-eighths of an inch.

Conduct steam through a small tube passing through the stopper and dipping deeply into the material in the flask. The distillation of the salicylic acid is facilitated by submerging the distilling flask almost to the stopper in an oil bath and distilling with the temperature of the oil at from 120° to 130°, or by adding about 20 grams of sodium chlorid to the contents of the flask for each 100 cubic centimeters of the substance, to raise the boiling point. Care must be taken not to let the contents of the flask get too low, as the heat will decompose the organic matter.

Collect at least 600 cubic centimeters of the distillate and continue the distillation until the last 200 cubic centimeters gives no color on the addition of a drop of ferric solution. The distilling apparatus should in all cases be tested with known amounts of salicylic acid in order to determine the amount of distillate necessary to carry over a definite weight of salicylic acid.

It is sometimes practicable to determine the salicylic acid directly in the distillate by the colorimetric method with ferric chlorid solution. If the mineral acid used in the distillation

be carried over mechanically, however, the accuracy of the method is greatly impaired. Salicylic acid may be recovered from the distillate after making alkaline and evaporating, if desired, by extraction with ether and estimating colorimetrically as directed below. Separate the clear aqueous solution, and if any emulsion is present give the separatory funnel a quick, vigorous shake and allow to settle again. If the emulsion is not broken up in this way, it may be accomplished by means of a centrifuge or by adding 10 or 15 cubic centimeters of low boiling point gasoline or petroleum ether and shaking again.

Separate the clear, aqueous portion obtained from the emulsion and add it to the first aqueous portion separated. Then pour the ether into another separatory funnel, care being taken that none of the aqueous portion is left with the ether. Return the aqueous portion to the separatory funnel and again extract with ether, following the same procedure as before. Repeat this operation twice, four separate extractions with ether being made in all.

In case of special difficulty in breaking up the emulsion in any of the extractions a small amount of ether may be allowed to remain with the aqueous portion rather than the reverse, as it is removed in successive extractions. Wash the combined ether extracts by shaking in a separatory funnel with one-tenth their volume of water (using, however, not less than 20 cubic centimeters of water at each washing). Care must be taken at each washing to separate the aqueous portion completely from the ether, but none of the ether should be allowed to run into the wash water.

Distil slowly the greater part of the ether, transfer the remainder to a porcelain dish, and allow to evaporate spontaneously. Thoroughly dry in a vacuum desiccator over sulfuric acid, extract the dry residue with 10 portions of 10 or 15 cubic centimeters each of carbon bisulfid or low boiling point petroleum ether, rubbing the contents of the dish with a glass rod or other suitable instrument and transferring the successive portions of solvent to a second porcelain dish. The extracted

residue should finally be tested with a drop of ferric-alum solution, and if any reaction for salicylic acid be given it should be taken up in water, re-extracted with ether, and the operation repeated. The gasoline extract is finally allowed to evaporate spontaneously. In examining a substance whose ether extract does not give a color or precipitate with ferric solution, the drying of the residue and its extraction with gasoline may be omitted. The residue may then be transferred by means of warm water directly from the distilling flask to the graduated flask, in which it is made up to a definite volume. Substances interfering with the ferric reaction may often be removed by precipitation with ferric chlorid or lime.

Dissolve the residue in a small amount of hot water and dilute to a definite volume. Dilute aliquots of the solution and match, in Nessler tubes or with a colorimeter, the color obtained by adding a few drops of ferric chlorid or ferric alum solution with that of a standard solution of salicylic acid containing about one milligram of salicylic acid in 50 cubic centimeters. A 0.5 per cent. solution of ferric chlorid should be used or a two per cent. solution of ferric alum. This solution should be boiled until a precipitate appears, allowed to settle, and filtered. The acidity of the solution is slightly increased in this manner, but so precipitated it keeps clear for a considerable time, and the turbidity caused by its dilution with water is much less and does not appear for a much longer time than if the unboiled solution is employed. This turbidity is especially objectionable in the quantitative estimation of salicylic acid, as it interferes with the exact matching of the color. In either case, and especially with ferric chlorid, an excess of reagent should be avoided, although an excess of 0.5 cubic centimeter of a two per cent. ferric alum solution may be added to 50 cubic centimeters of the solution of salicylic acid without impairing the results.

Salicylic acid may often be separated from fat extracted with the ether by washing the ether solution with dilute ammonium hydroxid. Then evaporate the aqueous liquid almost to dryness and test with ferric solution.

In the case of foods which yield to the gasoline solution of the ether residue a color that obscures the ferric chlorid reaction (for example, tomatoes), the ether solution may be evaporated, the residue dried in a desiccator or in a current of dry air, sublimed, and collected on a watch glass cooled with ice. Then dissolve the sublimate in hot water and test with ferric alum.

The same difficulty may often be avoided, and in fact the extraction with gasoline of the dry residue from the ether extraction may sometimes be obviated, by precipitating before extraction with ferric chlorid or calcium chlorid, making alkaline, and filtering. By this means tannin is entirely separated from the product and other substances whose color masks the salicylic acid reaction are often removed.

2. *Benzoic Acid*.—(a) *Qualitative Detection*.—Separate benzoic acid as directed for salicylic acid. If benzoic acid be present in considerable quantity, it will crystallize from the evaporated ether in shining leaflets with characteristic color on heating. Dissolve the residue in hot water, divide into two portions, and test by the following methods:

(1) *First Method*.⁹³—Make the residue alkaline with ammonium hydroxid, expel the excess of ammonia by evaporation, take up the residue with water, and add a few drops of a neutral 0.5 per cent. solution of ferric chlorid. The presence of benzoic acid will be indicated by the formation of a brownish-colored precipitate of ferric benzoate.

(2) *Second Method*.—Evaporate to dryness and treat the residue with two or three cubic centimeters of strong sulfuric acid. If this is the only method employed, the sulfuric acid may be added directly to the residue left on the evaporation of the ether. Heat until white fumes appear, organic matter is charred, and benzoic acid is converted into sulfo-benzoic acid. Then add a few crystals of potassium nitrate which causes the formation of meta-dinitrobenzoic acid. When cool dilute the acid with water and add ammonium hydroxid in excess. Then cool the mixture, transfer to a test tube, and add a drop or two of

⁹³ Mohler, Bul. soc. chim., Paris, 1890, 3 (3) : 414.

fresh, colorless ammonium sulfid so that the solutions do not mix. The nitrocompound is converted into ammonium meta-diamidobenzoic acid, which possesses a red color. This reaction takes place immediately and is seen at the surface of the liquid without stirring.

(b) *Quantitative Estimaton*.—Evaporate the ether extract obtained as directed under salicylic acid to dryness, thoroughly dry in a sulfuric acid desiccator (preferably in vacuum) and sublime under a watch glass cooled with a piece of ice or a condenser, the lower end of which is closed with a piece of rubber dam. Or the ether extract (or its solution in gasoline) may be transferred into the tube *a*, as shown in the accompanying

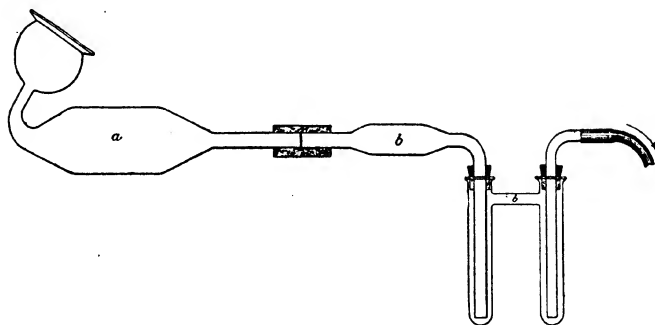


Fig. 118.—Apparatus for the Determination of Benzoic Acid.

figure, the ether or gasoline removed by a gentle current of air, the tube placed in a vacuum desiccator until its contents are thoroughly dry, and the residue sublimed at the temperature of 250° , the sublimate being collected in tube *b*.

During the sublimation, air is drawn very slowly through the apparatus (a wash bottle is used to gauge the speed of the current) to insure the volatilized benzoic acid passing into tube *b*. The joint between the two tubes is preferably made by means of a cork stopper. The most satisfactory results are obtained by placing the tube *a* inside of an oven the temperature of which is raised gradually until it reaches 250° . The bulb of the tube *b*

should be just outside of the oven, in order that the crystals may form therein. By means of this apparatus considerably higher results are obtained than by subliming on a watch glass, as described above.

The sublimate of benzoic acid collected in tube *b* may be removed by solution in alcohol, and the amount confirmed by titration. A sublimate is sometimes obtained which somewhat resembles benzoic acid in appearance and which has an acid reaction. Before applying the method, therefore, to any class of foods, blank experiments should be made to determine whether a sublimate is obtained under the same conditions from the ether extract of that class of foods.

3. *Saccharin*.—(a) *Qualitative Detection*.—Extract with ether (after maceration and exhaustion with water, if necessary), as described under salicylic acid. Allow the ether extract to evaporate spontaneously and note the taste of the residue. The presence of saccharin to the amount of 20 milligrams per liter is indicated by a sweet taste. This may be confirmed by heating with sodium hydroxid, as described below, and detecting the salicylic acid formed thereby. Results by this method indicating the presence of a faint trace of saccharin in wines which did not contain it have been frequently obtained, owing to the presence in wine of so-called “false-saccharin.”

Acidify 50 cubic centimeters of a liquid food (or the aqueous extract of 50 grams of a solid or semi-solid) and extract with ether. Test the extracted matter in the usual way for salicylic acid, return the gasoline extract to the dish containing the residue, dilute the whole to about 10 cubic centimeters volume, and add two cubic centimeters of sulfuric acid (1:3). Bring the solution to the boiling point and add a five per cent. solution of potassium permanganate, drop by drop, to slight excess; partly cool the solution, dissolve in it a piece of sodium hydroxid, and filter the mixture into a silver dish (silver crucible lids are well adapted to the purpose); evaporate to dryness and heat for 20 minutes at 210° to 215°. Dissolve the residue in water, acidify and extract with ether, evaporate the ether, and test the residue with

two drops of a two per cent. solution of ferric alum. By this method all the so-called false saccharin and the salicylic acid naturally present (also added salicylic acid when not present in too large amount) are destroyed, while five milligrams of saccharin per liter is detected with certainty.

8. *Sulfurous Acid*.—(a) *Qualitative Detection*.—To about 25 grams of the sample (with the addition of water, if necessary) placed in a 200 cubic centimeter Erlenmeyer flask, add some sulfur-free zinc, and several cubic centimeters of hydrochloric acid. In the presence of sulfites hydrogen sulfid will be generated and may be detected with lead paper. Results obtained should be verified by the distillation method.

It is always advisable to make the quantitative determination of sulfites, owing to the danger that the test may be due to traces of sulfids. A trace is not to be considered sufficient indication of the presence of sulfur dioxid either as a bleaching agent or as a preservative.

(b) *Determination of Total Sulfurous Acid*.—(1) *First Method*. —(*Distillation Method*).—Distil 100 grams (adding water, if necessary) in a current of carbon dioxid after the addition of about five cubic centimeters of a 20 per cent. solution of glacial phosphoric acid until 50 cubic centimeters have passed over. Collect the distillate in a tenth-normal iodine solution in a flask closed with a stopper perforated with two holes, through one of which the end of the condenser passes and through the other a U-tube containing a portion of the standardized iodine solution. Twenty-five cubic centimeters of tenth-normal iodine solution may be employed, diluted with water to give the desired volume. The method and apparatus may be simplified without material loss in accuracy by omitting the current of carbon dioxid, adding 10 cubic centimeters of phosphoric acid instead of five cubic centimeters, and dropping into the distilling flask a piece of sodium bicarbonate, weighing not more than a gram, immediately before attaching to the condenser. The carbon dioxid liberated is not sufficient to expel the air entirely from the apparatus, but will prevent oxidation to a large extent.

The U-tube trap may also be omitted if the end of the condenser tube is made to extend below the surface of the iodine solution, and the distillation conducted with a steady flame. When the distillation is finished wash the contents of the U-tube into the flask and determine the excess of iodine with standard thiosulfate solution. On account of its lack of permanence the iodine solution employed should be titrated from time to time with a tenth-normal thiosulfate solution (containing 24.8 grams $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ per liter). One cubic centimeter of tenth-normal iodine solution is equivalent to 0.0032 gram of sulfur dioxide (SO_2).

(2) *Second Method (Direct Titration Method).*—In the examination of wine fairly accurate results may also be obtained by the following method. Care must be taken in applying the method to other products than wine to determine whether iodine is decolorized by any substance that may be naturally present.

Place 25 cubic centimeters of a solution of potassium hydroxide containing 56 grams per liter in a flask of approximately 200 cubic centimeters capacity. Introduce 50 cubic centimeters of the sample by means of a pipette, mix with the potassium hydroxide, and allow the mixture to stand 15 minutes with occasional agitation. Add 10 cubic centimeters of 1:3 sulfuric acid and a few cubic centimeters of starch solution, and titrate the mixture with a fiftieth-normal iodine solution. Introduce the iodine solution as rapidly as possible and continue the addition until the blue color will last for several minutes. One cubic centimeter of fiftieth-normal iodine solution is equivalent to 0.00064 gram of sulfur dioxide.

(c) *Determination of Free Sulfurous Acid.*—(*Especially Adapted to Wine.*)—Treat 50 cubic centimeters of the sample in a flask having a capacity of approximately 200 cubic centimeters with about five cubic centimeters of 1:3 sulfuric acid, add a small piece of sodium carbonate (about 0.5 gram) to expel the air, and titrate the sulfurous acid with fiftieth-normal iodine solution, as directed under total sulfurous acid.

Gum and Dextrin.—*Official Method.*⁹⁴—Evaporate 100 cubic

⁹⁴ Bureau of Chemistry, U. S. Department of Agriculture, Bulletin 107 (revised), 1912: 87.

centimeters of wine to about 10 cubic centimeters and add 10 cubic centimeters of 96 per cent. alcohol. If gum or dextrin be present (indicated by the formation of a voluminous precipitate), continue the addition of alcohol slowly and with stirring until 100 cubic centimeters have been added. Let stand over night, filter and wash with 80 per cent. alcohol by volume. The precipitate may then be dried and weighed, or it may be treated according to one of the methods for the determination of starch.

678. Determination of Nitrogen.—The best method of determining nitrogen in fermented beverages is the common one of moist combustion with sulfuric acid. The sample is placed in the kjeldhal digestion flask, which is attached to the vacuum service and placed in a steam bath until its contents are dry or, nearly so. The process is then conducted in harmony with the well known methods. Where large quantities of the sample are to be employed, as in drinks containing but little nitrogen, the preliminary evaporation may be accomplished in an open dish, the contents of which are transferred to the digestion flask before any solid matter is deposited. In this case great care must be exercised to avoid loss of nitrogen from deposits that cling with great tenacity to the dish. The same procedure may be followed when the sample foams too much on heating.

679. Substitute for Hops.—It is often claimed that cheap and deleterious bitters are used in brewing in order to save hops. While it is doubtless true that foreign bitters are sometimes employed, experience shows that such an adulteration is not very prevalent in this country.⁹⁵ The only extensive substitute for hops appears to be in the use of lupulin, the principle bitter constituent of the hops. Possibly strychnin, picrotoxin, quassin, gelatin and other bitter principles have sometimes been found in beer, but their use is no longer common. It is difficult to decide in every case whether or not foreign bitters have been added. A common process is to treat the sample with lead acetate, filter, remove the lead from the filtrate and detect any

⁹⁵ Chemical Division, U. S. Department of Agriculture, Bulletin 13, 1887 : 296.

remaining bitters by the taste. All the hop bitters are removed by the above process. Any remaining bitter taste is due to other substances. For the methods of detecting the special bitter principles in hops and other substances, the work of Dragendorff may be consulted.⁹⁶

680. Bouquet of Fermented and Distilled Liquors.—The bouquet of fermented and distilled liquors is due to the presence of volatile matters which may have three different origins. In the first place the materials from which these beverages are made contain essential oils and other odoriferous principles. In the grape, for instance, the essential oils are found particularly in the skins. These essential principles may be secured by distilling the skins of grapes in a current of steam. This method of separation, however, cannot be regarded as strictly quantitative.

In the second place, the yeasts which produce the alcoholic fermentation are also capable of producing odoriferous products. These minute vegetations, resembling in their biological relations the mushrooms, grow in the soil and reach their maturity at about the time of the harvest of the grapes. Their spores are transmitted through the grapes or the air, reach the expressed grape juice and produce the vinous fermentation. The particular odor due to any given yeast persists for some time during successive cultures, showing that the body which produces the odor is the direct result of the vegetable activity of the yeast. A beer yeast will still give a product which smells like beer, and in like manner a wine yeast will produce one which has the odor of wine. The quantity of odorant matter produced by this vegetable action is so minute as to escape detection in a quantitative or qualitative way by chemical means. These subtle perfumes arise moreover not only from the breaking up of the sugar molecule, but are also the direct results of activities under the influence of the yeast itself.

In the third place, the fermented and distilled liquors contain odoriferous principles due to the chemical reactions which take

⁹⁶ Dragendorff, *Plant Analysis*, 1909 : 38 et. seq. Translated by H. G. Greenish.

place by the breaking up of the sugar and other molecules during the process of fermentation and aging. The alcohols and acids produced have distinct odors by which they are often recognized. This is particularly true of ethylic, propylic, butylic, amylic and oenanthylic alcohols and acetic acid. These alcohols themselves also undergo oxidation, passing first into the state of aldehyds which, together with ethers, produce the peculiar aroma which is found in various fruits. The etherification noted above is of course preceded by the formation of acids corresponding to the various aldehyds present. The formation of these ethers takes place very slowly during aging, and it therefore requires three or four years for the proper ripening of wines or distilled liquors. By means of artificial heat, electricity and aeration, the oxidizing processes above noted may be hastened, but it is doubtful whether the products arising from this artificial treatment are as perfect as those which are formed in the natural processes.

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